I U C L I D

Data Set

Existing Chemical	:	ID: 25637-99-4
Memo	:	HBCD
CAS No.	:	25637-99-4
EINECS Name	:	hexabromocyclododecane
EC No.	:	247-148-4
TSCA Name	:	Cyclododecane, hexabromo-
Molecular Formula	:	C12H18Br6

Producer related part

Company	:	ALBEMARLE CORPORATION
Creation date	:	30.11.2004

Substance related part

Company	:	ALBEMARLE CORPORATION
Creation date	:	30.11.2004
Status	:	
Memo	:	
Printing date	:	12.01.2005
Revision date	:	
Date of last update	:	12.01.2005
Number of pages	:	88
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	manufacturer
Name	:	ALBEMARLE CORPORATION
Contact person	:	Dr. M. Hardy
Date	:	30.11.2004
Street	:	451 Florida Street
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Homepage	:	

11.01.2005

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	HEXABROMOCYCLODODECANE
Smiles Code	:	c1cc(Br)c(Br)ccc(Br)c(Br)ccc(Br)c2(Br)
Molecular formula	:	C12H18Br6
Molecular weight	:	641.7
Petrol class	:	

30.11.2004

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	typical for marketed substance
Substance type	:	organic
Physical status	:	solid
Purity	:	>= 96 - 99.9 % w/w
Colour	:	white
Odour	:	odorless

Attached document : COMPOSITION OF THE COMMERCIAL HEXABROMOCYCLODODECANE (HBCD) PRODUCT

The commercial hexabromocyclododecane (HBCD) product contains three stereoisomers. The three isomers are typically referred to as alpha, beta and gamma based on their order of elution from a reverse phase HPLC column. The predominant isomer in the commercial product is the gamma stereoisomer. Based on analyses performed in support of toxicology

studies (described below), the commercial product consists of 80-85% gamma, 8-9% alpha, and 6% beta. The major impurity is tetrabromocyclododecene.

One manufacturer also produces a product of approximately 90% gamma HBCD content. However, the majority of the global market consists of the 80-85% gamma product.

The American Chemistry Council's Brominated Flame Retardant Industry Panel (BFRIP) sponsored a number of tests investigated the potential effects of HBCD. These tests were performed over the course of eight years. The test article used in these tests was a composite composed of equal parts of the members' commercial HBCD product. BFRIP members are Albemarle Corporation, Dead Sea Bromine Group and Great Lakes Chemical Corporation. Each test article was characterized in accordance with Good Laboratory Practices guidelines. The composition of the test article used in the various tests ranged from approximately 90 to 100% HBCD (Table 1). The impurities, when present, were those typically observed, e.g. tetrabromocyclododecene, isobutanol, and other unidentified compounds.

Tests performed from 2001-2003 typically utilized a test article of 99.9% purity (Table 1). The stereoisomer content of this test article was approximately 85% gamma, 9% alpha and 6% beta. Test article used in earlier years was comparable with a slightly lower gamma content (80% gamma, 8% alpha and 6% beta) (Table 1).

Table 1. COMPOSITION OF HBCD USED AS TEST ARTICLE IN BFRIP-SPONSORED STUDIES (all studies performed using a composite of equal parts of Albemarle Corporation, Dead Sea Bromine Group and Great Lakes Chemical Corporation commercial product)

Study	Year	Mean Isomer Content (Area %)			Isomer Sum (Purity)
		Alpha	Beta	Gamma	
Validation of Water Solubility Analytical Method	1997	8.5	6.0	79.1	93.6
Water Solubility	1997	8.5	6.0	79.1	93.6
Log Kow	1997	8.5	6.0	79.1	93.6
Vapor Pressure	1997	8.5	6.0	79.1	93.6
Fish LC50	1997	8.5	6.0	79.1	93.6
Daphnia EC50	1997	8.5	6.0	79.1	93.6
Freshwater Algae EC50	1997	8.5	6.0	79.1	93.6
Daphnia Chronic	1998	8.5	6.0	79.1	93.6
Fish Early Life Stage	2001	9.4	6.3	84.3	100
Fish Bioconcentration+	2000	6.4	4.5	79.1	90
Rat 28 Day	1996	8.5	6.0	79.1	93.6
Rat 90 Day	2001	8.9	6.6	84.5	99.99
Rat Developmental+					

1999 6.4 4.5 79.1 90
Guinea Pig Maximization
1997 8.5 6.0 79.1 93.6
Mouse LLNA*
2003 8.68 6.12 85.19 99.99
Chromosome Aberration
1996 8.5 6.0 79.1 93.6
In vivo Micronucleus
2000 8.9 6.6 84.5 99.99
Earthworm Survival & Reproduction*
2002 8.68 6.12 85.19 99.99
Terrestrial Plant: Seedling Emergence and Growth*
2002 8.68 6.12 85.19 99.99
Sediment Organism*
2003 8.68 6.12 85.19 99.99
Ready Biodegradability
1996 8.5 6.0 79.1 93.6
Sludge Respiration Inhibition*
2003 8.68 6.12 85.19 99.99
Soil Microcosm Biodegradation*
2003 8.68 6.12 85.19 99.99
Sediment Microcosm Biodegradation*
2003 8.68 6.12 85.19 99.99
Water Solubility of alpha, beta, gamma isomers
2004 7.67 5.15 83.04 95.86
Marine Algae, EC50
2004 7.67 5.15 83.04 95.86
*Results from reanalysis using stablilized THF; Initial analysis indicated 5.8% alpha isomer, 19.3% beta isomer, and 74.9% gamma isomer.
+Impurities specified as TetraBCDodecene 0.7%, Isobutanol 0.1%; Other Unknowns 9.2%

Flag : Critical study for SIDS endpoint
11.01.2005

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

cyclododecane, hexabromo-

30.11.2004

HBCD

28.12.2004

1.3 IMPURITIES

Purity : typical for marketed substance
CAS-No :
EC-No :
EINECS-Name : tetrabromocyclododecene
Molecular formula :
Value :

30.11.2004

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : industrial
Category : Polymers industry

30.11.2004

1.7.1 DETAILED USE PATTERN

Industry category : 11 Polymers industry
Use category : 22 Flame-retardants and fire preventing agents
Extra details on use category : Polymer processing
No extra details necessary
Emission scenario document : not available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

Attached document : HBCD is used solely as a flame retardant. Its primary application is in extruded (XPS) and expanded (EPS) polystyrene foam that is used as thermal insulation in the building industry. HBCD is highly efficient in this application so that very low levels are required to reach the desired flame retardancy. Typical HBCD levels in EPS are 0.67% and in XPS 2.5%. At present, HBCD is the only suitable flame retardant for these applications. Any other flame retardant would likely need higher load levels in the polystyrene foam.

A secondary, though important, application of HBCD is as a flame retardant for upholstery textiles. In this application, HBCD is applied to the back of the upholstery fabric encapsulated in a polymer. Typical HBCD levels in the polymer backcoat are 6-15%. The potential exposure and hazard to consumers associated with this use were reviewed recently by the U.S.

National Research Council (D. Gardner and B. Walker, Chair, Toxicological Risks of Selected Flame Retardants, 2000, National Academy Press, Washington, D.C.; http://www.nap.edu). The review found that direct exposure to the consumer was likely to be minimal, that the hazard index was less than 1 for all exposure routes (e.g. not likely to pose a health hazard), and that no further research was needed for assessing consumer health risks from HBCD.

A very minor application for HBCD is in video or audio equipment housings where V-2 levels of flame retardancy are acceptable. HBCD is not used to flame retard electronic housings (e.g. television sets) that must meet the higher V-0 flame retardancy standard.

28.12.2004

1.7.2 METHODS OF MANUFACTURE

Origin of substance : Synthesis
Type :

30.11.2004

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

Type : EINECS
Additional information : TSCA; Japan

28.12.2004

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

Type of search : External
Chapters covered :
Date of search : 15.12.2004

Attached document : A search for all documents containing the word "hexabromocyclododecane" was performed using PubMed. The search produced 20 hits.

15.12.2004

1.13 REVIEWS

2.1 MELTING POINT

Decomposition	:	yes, at ca. 269 - 279 °C
Sublimation	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Attached document	:	The commercial HBCD has a melt range of 180-185 degrees Centigrade. TGA analysis of the commercial HBCD product indicates 50% weight loss at 269 degrees Centigrade and 90% weight loss at 274 degrees Centigrade.

28.12.2004 (1)

2.2 BOILING POINT

2.3 DENSITY

2.3.1 GRANULOMETRY

Type of distribution	:	other
Precentile	:	
Attached document	:	HBCD used in extruded or expanded polystyrene (predominant application) is sold either as granules (> 1 mm) or large particles (mean diameter 110 microns). For use in textile applications (lesser application), HBCD is ground to a fine particle size in order to ease its dispersion in coatings. The average particle size for textile-grade-HBCD is 1.98 microns.

27.12.2004

2.4 VAPOUR PRESSURE

Value	:	= .0000627 hPa at 21 °C
Decomposition	:	no
Method	:	OECD Guide-line 104 "Vapour Pressure Curve"
Year	:	1997
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	US EPA OPPTS 830.7950. Determined using the spinning rotor gauge.
Remark	:	Sponsor: American Chemistry Council (ACC) Brominated Flame Retardant Industry Panel (BFRIP) Sponsor: American Chemistry Council (ACC) Brominated Flame Retardant Industry Panel (BFRIP)
Reliability	:	(1) valid without restriction
Flag	:	Risk Assessment, Critical study for SIDS endpoint

28.12.2004 (2)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= 5.625 at 25 °C
pH value	:	
Method	:	other (measured)
Year	:	1997
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	US EPA OPPTS 830.7560. Gnenerator column method.
Remark	:	Sponsor: American Chemistry Council Brominated Flame Retardant Industry Panel (BFRIP).
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

30.11.2004

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 3.4 other: ug/L at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	yes
Deg. product	:	no
Method	:	OECD Guide-line 105
Year	:	1997
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Sponsor: ACC Brominated Flame Retardant Industry Panel
Attached document	:	HBCD's water solubility using the generator column/column elution method (EPA 796.1860)/OECD 105) was determined to be 3.4 ug/L at 25 degrees C. The analytical method, HPLC using a UV dectector, was validated prior to use (Kendall and Nixon. Analytical method verification for the determination of hexabromocyclododecane (HBCD) in well water. Final Report. Wildlife International LTD. Project Number: 439C-107. Wildlife International LTD, Easton, MD). This method, the most sensitive available at the time of study performance, was capable of quantitating the gamma isomer, only. UV-active substances, whose peaks could not be resolved, interfered with quantifying the alpha and beta content. The solubility of HBCD was therefore based on that of the gamma isomer, which comprised ~80% of the test article. The results of this water solubility study were used to set dose levels for aquatic studies performed between 1997 and 2001, and sponsored by ACC Brominated Flame Retardant Industry Panel.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

16.12.2004 (3)

Solubility in	:	Water
Value	:	ca. 2.08 - 48.8 other: ug/L at 20 °C
pH value	:	
concentration	:	at °C

Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	yes
Deg. product	:	no
Method	:	OECD Guide-line 105
Year	:	2004
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Remark : Sponsor: ACC Brominated Flame Retardant Industry Panel

Attached document :

HBCD: WATER SOLUBILITY OF THE THREE DIASTEROMERS

A study, which was performed according to current OECD/EPA guidelines and Good Laboratory Practices (GLPs), determined HBCD's water solubility to be 3.4 ug/L using a generator column with HPLC/UV as the analytical method (Stenzel and Markley, 1997). The test article was a composite of the 3 manufacturers' commercial product and its composition was 79.1% gamma, 6.0% alpha and 8.5% beta. The best analytical method available at that time was used; e.g. HPLC with a UV detector. That method was able to quantify the gamma isomer. UV-active substances, whose peaks could not be resolved, interfered with quantifying the alpha and beta content. Thus, the solubility of the commercial product was based on that of the gamma isomer. This was a reasonable approach, given that the technical product was largely composed (~80%) of the gamma isomer.

In late 2003, further studies were undertaken to determine HBCD's potential effects on the marine algae, Skeletonema, in order to clarify results reported in Walsh et al., 1987. Preliminary work for the 2003 algae study (Desjardins et al. 2003) investigated whether HBCD exposures in marine media would be best conducted utilizing the water accommodated fraction (WAF) or eluates produced from a generator column. The analytical method utilized was HPLC/MS, an evolution of the original method that allowed quantification of all 3 isomers. The WAF preparations had serious problems with HBCD particulates that could not be resolved in a consistent manner by filtering or centrifugation. Thus, it was concluded the WAF method was not appropriate. The HBCD eluate from the generator column solved the particulate problem, but also revealed that the water solubility of the alpha and beta isomers was significantly different than that of the gamma isomer. The water solubility of the gamma isomer in the marine media was nearly equivalent to that determined in 1997 with reagent water. The solubility of the alpha and beta isomers was approximately 47 and 16 ug/L, respectively. A standard GLP solubility study (MacGregor and Nixon. 2004) using reagent water confirmed these results. Thus, under the conditions of the generator column, HBCD's total water solubility (expressed as the sum of the three isomers) was 65.6 ug/L, and that of the gamma, alpha and beta isomers was 2.08, 48.8 and 14.7 ug/L, respectively.

Desjardins D, MacGregor J and Krueger H. 2003. Draft Report: Hexabromocyclododecane (HBCD): A 72-Hour Toxicity Test with the Marine Diatom (Skeletomema costatum). Wildlife International Project Number: 439A-125. Wildlife International, Ltd. Easton, MD.

MacGregor J and Nixon W. 2004. Final Report. Determination of Hexabromocyclododecane (HBCD) Diastereomers using a Generator Column Method. Wildlife International Project Number: 439C-138. Wildlife International, Ltd. Easton, MD.

Stenzel J. and Markley B. 1997. Final Report. Hexabromocyclododecane (HBCD): Determination of the Water Solubility. Wildlife International Project Number: 439C-105. Wildlife International, Ltd. Easton, MD.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
20.12.2004 (4)

Deg. product :
Method : other
Year : 2000
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Attached document : A technical data sheet from Albemarle Corporation (accessed 12/2002) reports the following solubilites for HBCD:

Solubility (weight % at 25°C)
Water..... < 0.01
Acetone 8.6
Methanol 0.15
Toluene..... 6.40
n-Pentane..... 0.01
Isopentane 0.01
Cyclopentane..... 0.05
Styrene..... 8.00
Chlorobenzene..... 2.80
Methylene dibromide..... 3.60
Dimethyl formamide 33.90

27.12.2004 (1)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

3.1.2 STABILITY IN WATER

3.1.3 STABILITY IN SOIL

Type	:	other: laboratory, aerobic
Radiolabel	:	no
Concentration	:	.025 mg/kg
Soil temperature	:	20 °C
Soil humidity	:	20 other: %
Soil classification	:	USDA
Year	:	2003
Content of clay	:	= 16 %
Content of silt	:	= 20 %
Content of sand	:	= 64 %
Organic carbon	:	= 1.8 %
pH	:	= 6.4
Cation exch. capacity	:	= 19.2 meq/100 g soil dry weight
Microbial biomass	:	= 274 other: ug/g dry weight soil
Dissipation time		
DT50	:	= 63 day(s)
DT90	:	
Dissipation	:	= 75 % after 119 day(s)
Deg. product	:	
Method	:	other: OECD 307
Year	:	2003
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : HBCD Transformation in Aerobic and Anaerobic Soil Microcosms (Sponsor: ACC Brominated Flame Retardant Industry Panel and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. Its composition was 8.68% alpha isomer, 6.12% beta isomer and 85.19% gamma isomer. This study was performed according to Good Laboratory Practices and OECD 307.

The transformation of HBCD was determined in aerobic and anaerobic soils based on the Organization for Economic Co-Operation and Development (OECD) Test Guideline 307 "Aerobic and Anaerobic Transformation in Soil." Soil microcosms were prepared by adding a sandy loam surface soil to serum bottles sealed with Teflon coated septa. Aerobic microcosms were prepared by adjusting the soil moisture to 20% (by weight) and periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen. The microcosms were pre-incubated at 20 ± 1 °C for 35 days. Anaerobic microcosms were prepared in an anaerobic atmosphere (70% N2, 28% CO2, and 2% H2) by flooding the soil with water and pre-incubating the microcosms at 23 ± 1 °C for 43 days to allow low redox (e.g., methanogenic) conditions to develop. HBCD was then added to microcosms at a nominal concentration of 25 ng/g (soil dry weight), together with activated sludge (5 mg/g, dry weight basis) from a municipal wastewater treatment plant to simulate sludge land treatment applications. Biologically inhibited (abiotic) controls were prepared by

steam sterilization prior to the addition of HBCD. Microcosms were incubated in the dark at 20 ± 1 °C for 119 days. The concentration of HBCD in the microcosms was determined at selected time intervals utilizing high performance liquid chromatography-mass spectrometry (LC-MS). Aerobic microcosms were analyzed on days 0, 1, 7, 21, 48, 65, and 119, while anaerobic microcosms were analyzed on days 0, 1, 7, 21, 56, 91, and 119.

HBCD concentrations decreased over time in both the aerobic and anaerobic soils. HBCD concentrations decreased 75% over 119 days in the viable aerobic soil microcosms, compared to a 3% decrease in the abiotic controls, indicating that biological processes were responsible for most of the losses observed. Under anaerobic conditions, HBCD concentrations decreased 92% over 21 days in the viable microcosms compared to a less than 1% decrease in the abiotic controls. Pseudo-first order kinetics rate constants for the biotransformation of HBCD were determined by subtracting the abiotic rate constant from the viable rate constant. Biotransformation half-lives for HBCD were determined to be 63 and 6.9 days in the aerobic and anaerobic soils, respectively. Brominated degradation products were not detected in the soil or in the headspace of the microcosms.

The purpose of this study was to determine the environmental lifetime of HBCD under realistic environmental conditions. Laboratory microcosms were used to evaluate the transformation of HBCD in aerobic and anaerobic soils. Soil degradation processes are expected to play a major role in determining the environmental lifetime of HBCD since, upon release into the environment, the majority of HBCD is likely to partition into the soil or sediment compartments. In this study, HBCD loss was observed in both viable and abiotic soil microcosms although the rates were appreciably faster in the viable soils. Biologically mediated transformation processes (i.e., biotransformation) accelerated the rate of loss of HBCD when compared to the biologically inhibited (i.e., heat-treated) soils. No brominated degradation products were observed in either system.

Limited information is available for the reactions of HBCD in the environment. However, the aerobic degradation and mineralization of a similar type of cyclic, aliphatic halogenated fire retardant, FR-651A (mixture of pentabromochlorocyclohexane, tetrabromo-dichlorocyclohexane, and tribromotrichlorocyclohexane) was observed. A soil half-life of ~11 days based upon disappearance of 14 C-FR-651A from soil was reported. Complete degradation of 14C-FR-651A was also observed with mineralization half-life on the order of 93 days.

An examination of the reactions of brominated aliphatic compounds that contain structural features similar to HBCD can also provide insight into the possible reaction pathways available for HBCD. Brominated aliphatic compounds are known to be susceptible to both hydrolytic and nuclelophilic attack. The reactivity of halogenated aliphatic compounds increases in the order of F < Cl < Br. For example, the hydrolysis half-lives at pH 7 and 25 °C for methyl fluoride, methyl chloride, and methyl bromide are 1.1 x 104, 340, and 20 days, respectively. At environmental pH's, neutral and base catalyzed hydrolysis reactions are most important, and depend on the degree and patterns of halogen substitution.

Sulfur based nucleophiles can react rapidly with halogenated aliphatic compounds, thereby reducing their lifetimes in the environment. The half-life of 1-bromohexane in water at 25 °C was reduced from 20 days to approximately one day in 5 mM HS - or 0.07
mM polysulfide (Sx 2-). Similarly, the half-life of 1,2-dibromoethane in water at 25 °C was reduced from 1,000 days to 4 days in the presence of 5 mM HS-. Sulfide and polysulfides would be expected to be present in soils

at low redox potentials. Thus, reaction of HBCD with these sulfur-based nucleophiles may partly explain the loss of HBCD in the soil studies.

Since HBCD contains three pairs of vicinal bromine atoms, the transformation of simple aliphatic compounds containing vicinal bromine atoms may provide insight into possible reaction pathways for HBCD. For example, 1,2-dibromoethane reacts with nucleophiles via both substitution and elimination reactions. Reaction with HS- via an "SN2" substitution reaction results in the formation of HS-CH2-CH2-SH, while an elimination reaction under alkaline conditions results in the formation of H2C=CHBr. A combination of elimination and substitution reactions can result in the formation of a mixture of HO-H2-CH2-OH and H2C=CHBr. Similar mechanisms may be responsible for the loss of HBCD observed in this study.

Halogenated aliphatic compounds are susceptible to reductive dehalogenation reactions in natural reducing environments. The rates of reactions vary depending on the strength and electron affinity of the carbon-halogen bond and the stability of the carbon-radical species resulting from the initial electron transfer that occurs. Vicinal dehalogenation reactions are particularly important, and are dependent on the halogen atom. Half-lives for vicinal dehalogenation of 1,2-diiodoethane, 1,2-dibromoethane, and 1,2-dichloroethane are 0.6, 55, and 1.8 x 104 hours, respectively. The rapid disappearance of HBCD in the anaerobic soil microcosms may be partly explained by reductive dehalogenation reactions.

Davis J, Gonsior S and Marty G. 2003. Evaluation Of Aerobic And Anaerobic Transformation Of Hexabromocyclododecane In Soil. Study Number 021082. Environmental Chemistry Research Laboratory. Toxicology & Environmental Research and Consulting. The Dow Chemical Company. Midland, MI.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.01.2005 (5)

Type	:	other: laboratory, anaerobic
Radiolabel	:	no
Concentration	:	.025 mg/kg
Soil temperature	:	20 °C
Soil humidity	:	20 other: %
Soil classification	:	USDA
Year	:	2003
Content of clay	:	= 16 %
Content of silt	:	= 20 %
Content of sand	:	= 64 %
Organic carbon	:	= 1.8 %
pH	:	= 6.4
Cation exch. capacity	:	= 19.2 meq/100 g soil dry weight
Microbial biomass	:	= 274 other: ug/g dry weight soil
Dissipation time		
DT50	:	= 6.9 day(s)
DT90	:	
Dissipation	:	= 92 % after 21 day(s)
Deg. product	:	
Method	:	other: OECD 307
Year	:	2003
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : Transformation in Aerobic and Anaerobic Soil Microcosms (BFRIP and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. Its composition was 8.68% alpha isomer, 6.12% beta isomer, and 85.19% gamma isomer. This study was performed according to Good Laboratory Practices and OECD 307.

The transformation of HBCD was determined in aerobic and anaerobic soils based on the Organization for Economic Co-Operation and Development (OECD) Test Guideline 307 "Aerobic and Anaerobic Transformation in Soil." Soil microcosms were prepared by adding a sandy loam surface soil to serum bottles sealed with Teflon Ò coated septa. Aerobic microcosms were prepared by adjusting the soil moisture to 20% (by weight) and periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen. The microcosms were pre-incubated at 20 ± 1 °C for 35 days. Anaerobic microcosms were prepared in an anaerobic atmosphere (70% N2, 28% CO2, and 2% H2) by flooding the soil with water and pre-incubating the microcosms at 23 ± 1 °C for 43 days to allow low redox (e.g., methanogenic) conditions to develop. HBCD was then added to microcosms at a nominal concentration of 25 ng/g (soil dry weight), together with activated sludge (5 mg/g, dry weight basis) from a municipal wastewater treatment plant to simulate sludge land treatment applications. Biologically inhibited (abiotic) controls were prepared by steam sterilization prior to the addition of HBCD. Microcosms were incubated in the dark at 20 ± 1 °C for 119 days. The concentration of HBCD in the microcosms was determined at selected time intervals utilizing high performance liquid chromatography-mass spectrometry (LC-MS). Aerobic microcosms were analyzed on days 0, 1, 7, 21, 48, 65, and 119, while anaerobic microcosms were analyzed on days 0, 1, 7, 21, 56, 91, and 119.

HBCD concentrations decreased over time in both the aerobic and anaerobic soils. HBCD concentrations decreased 75% over 119 days in the viable aerobic soil microcosms, compared to a 3% decrease in the abiotic controls, indicating that biological processes were responsible for most of the losses observed. Under anaerobic conditions, HBCD concentrations decreased 92% over 21 days in the viable microcosms compared to a less than 1% decrease in the abiotic controls. Pseudo-first order kinetics rate constants for the biotransformation of HBCD were determined by subtracting the abiotic rate constant from the viable rate constant. Biotransformation half-lives for HBCD were determined to be 63 and 6.9 days in the aerobic and anaerobic soils, respectively. Brominated degradation products were not detected in the soil or in the headspace of the microcosms.

The purpose of this study was to determine the environmental lifetime of HBCD under realistic environmental conditions. Laboratory microcosms were used to evaluate the transformation of HBCD in aerobic and anaerobic soils. Soil degradation processes are expected to play a major role in determining the environmental lifetime of HBCD since, upon release into the environment, the majority of HBCD is likely to partition into the soil or sediment compartments. In this study, HBCD loss was observed in both viable and abiotic soil microcosms although the rates were appreciably faster in the viable soils. Biologically mediated transformation processes (i.e., biotransformation) accelerated the rate of loss of HBCD when compared to the biologically inhibited (i.e., heat-treated) soils. No brominated degradation products were observed in either system.

Limited information is available for the reactions of HBCD in the environment. However, the aerobic degradation and mineralization of a similar type of cyclic, aliphatic halogenated fire retardant, FR-651A (mixture of pentabromochlorocyclohexane, tetrabromo-dichlorocyclohexane, and

tribromotrichlorocyclohexane) was observed. A soil half-life of ~11 days based upon disappearance of 14 C-FR-651A from soil was reported. Complete degradation of 14C-FR-651A was also observed with mineralization half-life on the order of 93 days.

An examination of the reactions of brominated aliphatic compounds that contain structural features similar to HBCD can also provide insight into the possible reaction pathways available for HBCD. Brominated aliphatic compounds are known to be susceptible to both hydrolytic and nuclelophilic attack. The reactivity of halogenated aliphatic compounds increases in the order of F < Cl < Br. For example, the hydrolysis half-lives at pH 7 and 25 °C for methyl fluoride, methyl chloride, and methyl bromide are 1.1 x 104, 340, and 20 days, respectively. At environmental pH's, neutral and base catalyzed hydrolysis reactions are most important, and depend on the degree and patterns of halogen substitution.

Sulfur based nucleophiles can react rapidly with halogenated aliphatic compounds, thereby reducing their lifetimes in the environment. The half-life of 1-bromohexane in water at 25 °C was reduced from 20 days to approximately one day in 5 mM HS - or 0.07
mM polysulfide (Sx 2-). Similarly, the half-life of 1,2-dibromoethane in water at 25 °C was reduced from 1,000 days to 4 days in the presence of 5 mM HS-. Sulfide and polysulfides would be expected to be present in soils at low redox potentials. Thus, reaction of HBCD with these sulfur-based nucleophiles may partly explain the loss of HBCD in the soil studies.

Since HBCD contains three pairs of vicinal bromine atoms, the transformation of simple aliphatic compounds containing vicinal bromine atoms may provide insight into possible reaction pathways for HBCD. For example, 1,2-dibromoethane reacts with nucleophiles via both substitution and elimination reactions. Reaction with HS- via an "SN2" substitution reaction results in the formation of HS-CH2-CH2-SH, while an elimination reaction under alkaline conditions results in the formation of H2C=CHBr. A combination of elimination and substitution reactions can result in the formation of a mixture of HO-H2-CH2-OH and H2C=CHBr. Similar mechanisms may be responsible for the loss of HBCD observed in this study.

Halogenated aliphatic compounds are susceptible to reductive dehalogenation reactions in natural reducing environments. The rates of reactions vary depending on the strength and electron affinity of the carbon-halogen bond and the stability of the carbon-radical species resulting from the initial electron transfer that occurs. Vicinal dehalogenation reactions are particularly important, and are dependent on the halogen atom. Half-lives for vicinal dehalogenation of 1,2-diiodoethane, 1,2-dibromoethane, and 1,2-dichloroethane are 0.6, 55, and 1.8 x 104 hours, respectively. The rapid disappearance of HBCD in the anaerobic soil microcosms may be partly explained by reductive dehalogenation reactions.

Davis J, Gonsior S and Marty G. 2003. Evaluation Of Aerobic And Anaerobic Transformation Of Hexabromocyclododecane In Soil. Study Number 021082. Environmental Chemistry Research Laboratory. Toxicology & Environmental Research and Consulting. The Dow Chemical Company. Midland, MI.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.01.2005 (5)

06.12.2004

06.12.2004

3.2.1 MONITORING DATA

Type of measurement : background concentration
Media : other: biota, sewage treatment plant, sediment, landfill leachate
Concentration :
Method :

Attached document : HEXABROMOCYCLODODECANE ENVIRONMENTAL MONITORING: RESULTS OF ISOMER-SPECIFIC ANALYSIS AVAILABLE AS OF MAY 2004

Hexabromocyclododecane (HBCD) is used to flame retard extruded and expanded polystyrene that is then used as thermal insulation in buildings. A minor use is in upholstery textiles where it is applied as a backcoat on the fabric. The commercial HBCD product is composed of three stereoisomers that are designated alpha, beta and gamma in deference to their respective elution from a reverse phase column. The isomer content of the commercial product is typically 80-85% gamma, 8-9% alpha and 6% beta. The major impurity is tetrabromocyclododecene.

A laboratory fish bioconcentration study showed that the three stereoisomers were present in rainbow trout in rough approximation to that of the commercial product used as test article. A rat 90-day subchronic study on the commercial product showed that the alpha isomer predominated in adipose tissue with lesser quantities of the gamma and beta isomers. Given these apparent differences between species and isomers, the literature and unpublished data were reviewed for environmental monitoring studies that included HBCD (see Table below). Only those studies that utilized analytical methods allowing detection of the three HBCD isomers were included. Studies reporting only 'total HBCD' content were discarded. Results were separated by matrix: sewage treatment plants, sediment, landfill leachate, whole organisms, and specific tissues. The resulting dataset both as a whole and for any specific matrix/organism is small. Thus, caution should be used to guard against over interpretation of the results.

Sewage Treatment Plants (STP)
STPs appear to do a good job removing HBCD from the influent. Effluent from plants with detectable amounts of HBCD in the influent typically had no detectable HBCD. HBCD appears to settle in sludge; the gamma isomer typically represented some 50% of the total. Sludge from the UK and Ireland typically contained all three isomers, but only one STP in the Netherlands had detectable levels of the beta isomer in sludge. For the 5 UK plants with measured influent, 1 had no detectable HBCD in the influent, 3 had gamma but no alpha or beta in the influent, and 1 had all 3 isomers with beta predominating. In the Netherlands, for the 5 plants measured, 3 had alpha in the influent but not beta or gamma, one had predominantly gamma with lesser alpha, and one had no detectable HBCD.

Conclusion: STPS perform their function in removing HBCD from the influent. Effluents from plants receiving HBCD typically contained no detectable HBCD. Gamma typically made up approximately 50% of the total in sludge; e.g. the gamma predominated. The predominant isomer in the influent appears to vary with country (gamma in UK, alpha in the Netherlands)/ however, the dataset is extremely small and variable so this should be viewed with caution.

Sediment
In those sediments with detectable HBCD, the gamma isomer was detected with greatest frequency (9 out of 27 sediment samples from the Scheldt and Dutch Rivers had gamma as the predominant isomer and many of these had detectable levels of gamma only). Two sediments out of 27 had roughly equal levels of gamma and beta, one had roughly equal levels of alpha and gamma, and one had alpha only.

Conclusion - the HBCD isomer most often detected in sediment was gamma.

Landfill Leachate
Samples from the UK and Ireland had no detectable HBCD. Eleven samples of landfill leachate originating in the Netherlands were analyzed. Four contained no detectable HBCD. In the seven where HBCD was detected, the gamma isomer was the predominant and most frequently detected isomer. The beta isomer was detected in only one of the samples from the Netherlands.

Whole Organisms
Both alpha and gamma were detected in all Lake Ontario trout analyzed (5/5). Beta was not detected in any. Composites of 3 different feeder fish from Lake Ontario also had detectable levels of both alpha and gamma, but no beta. Composites of 3 invertebrates, analyzed separately by species, from Lake Ontario also had detectable levels of both alpha and gamma, but no beta. Alpha was present in the highest quantities in all Lake Ontario organisms. North Sea whelk, sea star and hermit crabs had no detectable levels of HBCD.

Conclusion - The alpha and gamma isomers were detected with the same frequency in freshwater species of Lake Ontario. The alpha isomer typically represented approximately 80% of the total. Analysis of whole organism, as opposed to individual tissues (see below), appears to generate the most consistent data.

Specific Tissues
Liver, bird. The alpha isomer was most frequently detected (and the predominant isomer) in cormorant liver (n=5).
Liver, marine mammal. One marine mammal liver contained alpha but not beta or gamma, and one contained no detectable HBCD (n=2). Thus, no conclusion can be suggested.
Muscle, fish. The dataset for Whiting is too small and variable to draw any conclusion. Whitefish (Switzerland) contained alpha, but no beta or gamma.
Muscle, bird. The alpha, beta and gamma isomers were detected with roughly equal frequency.
Egg, bird. The alpha isomer was most frequently detected. The numbers of eggs with detectable quantities of beta and gamma were roughly equal.
Blubber. The alpha isomer was the most frequently detected and typically in the highest quantity. Only one sample out of 11 had detectable amounts of all 3 isomers.
Eel. The alpha isomer was the most frequently detected and typically made up the largest proportion of the total. However, a reasonable number of samples also contained gamma, and in few cases, the alpha and gamma levels were approximately equal.

Conclusion. This dataset is extremely small and highly variable. Continued analysis of liver, muscle and eel does not appear justified. Egg and blubber may be good indicators for birds and marine mammals, and thus continued analysis of these tissues may prove useful.

Textile Plants Located in Europe
The gamma isomer was the predominant HBCD isomer detected in waste water from 4 of 5 textile plants. In 1 of these 5 plants, alpha and gamma were present in approximately equal quantities. The gamma isomer

predominated in soil surrounding the 5 plants.

SUMMARY
The published literature and unpublished data were reviewed for environmental monitoring results pertaining to HBCD. Only those studies that included specific analysis for the alpha, beta and gamma HBCD stereoisomers were included. Environmental matrixes reporting results for all three stereoisomers included sewage treatment plants, sediment, whole organisms, specific tissues, and textile plant wastewater, and textile plant soil. The gamma isomer predominated in sewage treatment plant sludge, sediment, landfill leachate, textile plant wastewater and textile plant soil. The alpha isomer was the predominant isomer detected on the analysis of whole organisms (lake trout, feeder fish, 3 freshwater invertebrates). Analysis of individual tissues produced varying results. Of the individual tissues analyzed, avian egg and marine mammal blubber appeared to produce the most consistent results. The alpha isomer was the most frequently detected isomer in both. Continued monitoring of egg and blubber may be of value, but other tissue types are not recommended. The most consistent data is apparently generated on the basis of whole organism analysis. Sewage treatment plants are effective in removing HBCD from the influent - effluents from plants receiving HBCD typically contained no detectable HBCD.

TABLE: HBCD ISOMER SPECIFIC ANALYSIS

A. In Various Tissues (Data from draft EU RA 2003, Gerecke 2003 and de Boer's bird study 2004)

	alpha	beta	gamma	
Liver (All ppb ww)				
Cormarant (ug/kg ww)				
	21	3	2	% Lipid not stated
		6	1	2
		33	1	1
		7	0.3	0
		2	0	0
H. Porpoise (ng/g ww)				
	2400	0	0	36% lipid, n=1
H. Seal		(ng/g ww)		
	0	0	0	2% lipid, n=2 or 3
Muscle OR Fillet (All ppb ww)				
Fish				
Whiting, UK (ug/kg ww)				
		506	247	283 % Lipid not staed
		81	61	149
		0	0	0
Whiting, Netherlands (ng/g ww)				
		0	0	0 0.6% Lipid, n=3
Whitefish, Switzerland, (ng/g lw)				
		210	0	0 2.6%., pool of 10
		100	0	0 3.8%, pool of10
		66	0	0 1.5%, pool of 10
		54	0	0 7.2%, pool of 10
Avian				
Sparrow Hawk (ug/kg ww)				
		61	0	0 2.6% Lipid
		0	8.1	19 1.3
		0	9.4	11 2.2
		2.6	0	0 3.1
		13	26	150 1

	0	7.1	0	0.8
	4.8	0	0	1
	2.6	0	0	1.1
	0	9.2	0	1
	0	0	0	0 - 8.5% LIPID, n=60

Egg, Avian (ug/kg ww)

Falcon				
	38	0	0	7
	72	0	0	11.1
	22	0	0	7.7
	0	9.2	0	0.8
	13	0	0	7.3
	5.1	0	0	7.2
	0	3.4	4.6	6.4
	28	0	0	5.5
	30	13	12	7.1
	0	8.9	0	6.3
	15	0	5.5	6.3
	27	0	0	4.2
	20	0	0	5.0
	0	0	0	0.8-17.1; n=40

Blubber (All ppb ww)

Porpoise (ug/kg ww)			
298	302	317	% Lipid not stated, n=5 f
	315	2	5
	53	1	0
	89	0.4	0
	0	0	0

H. Porpoise (ng/g ww)

3500	0	0	90% Lipid, n=4
	410	0	0
	6400	0	0
	800	0	0

H. Seal (ng/g ww)

2000	0	0	70% Lipid, n=2
	0	0	0

Eel (whole body?) (ug/kg ww)

0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
25	5	11
25	0	8
5	0	0
0	0	0
22	21	1
2	0	0
5	0	0
3	0	0
0	0	0
0	0	0
52	0	12
36	0	8
28	0	7

8	0	0
14	0	0.4
17	0	15
2	0	0
4	0	0
8	0	10

B. WHOLE ORGANISMS (Data from Tomy et al 2004 and de Boer re North Sea)

	Alpha	Beta	Gamma	%Lipid, n
Lake Trout, Lake Ontario, whole fish (ng/g ww), n=5				
	1	0	0.3	11 % lipid
	0.4	0	0.07	14% lipid
	1.2	0	0.26	9 % lipid
	0.6	0	0.1	15 % lipid
	3.8	0	0.7	14% lipid
Rainbow Smelt, Lake Ontario, composites of whole fish (ng/g ww)				
	0.3	0	0.04	1.4% lipid, n=5 fish
	0.2	0	0.04	1.6% lipid, n=5 fish
	0.2	0	0.03	1% lipid, n=20 fish
Slimy sculpin, Lake Ontario, composites of whole fish (ng/g ww)				
	0.1	0	0.02	0.9, n=15
	0.4	0	0.1	3%, n=10
	0.4	0	0.1	2%, n=10
Alewife, Lake Ontario, composites of whole fish (ng/g ww)				
	0.15	0	0.02	3.5% lipid, n=5
	0.12	0	0.01	5.5% lipid, n=5
	0.08	0	0.01	1.5% lipid, n=5
Mysis, Lake Ontario, composite of whole organisms (ng/g ww)				
	0.07	0	0.02	3.1 % lipid, n>100
	0.04	0	0.01	3.8% lipid, n>100
Diporeia, Lake Ontario, composite of whole organisms (ng/g ww)				
	0.06	0	0.03	1.7%lipid, n>100
	0.05	0	0.02	1%lipid, n>100
Plankton, Lake Ontario, composite of whole organisms (ng/g ww)				
	0.04	0	0.03	0.6% lipid
	0.02	0	0	0.2% lipid
Whelk, North Sea (ng/g ww)				
	0	0	0	2.4%
	0	0	0	1.8
	0	0	0	1.5
Sea Star, North Sea (ng/g ww)				
	0	0	0	3.5%
	0	0	0	7.5
	0	0	0	7.6
Hermit Crab, North Sea (ng/g ww)				
	0	0	0	7.4%
	0	0	0	6.7
	0	0	0	8.5
	0	0	0	10.6
	0	0	0	9
	0	0	0	7.3
	0	0	0	9.5
	0	0	0	14.1
	0	0	0	9.8

C. SEWAGE TREATMENT PLANTS (Data from de Boer et al., 2002; as reported in draft EU Risk Assessment 2003)

	Alpha	Beta	Gamma
Burnham, UK			
Influent dissolved phase			

7.9	12.5	3.2	ng/L		
Influent particulate phase					
	0	29.4	0	ug/kg dw	
Sludge		132	458	666	ug/kg dw
Effluent dissolved phase					
	0	0	0	ng/L	
Effluent particulate phase					
	0	0	0	ug/kg dw	
Latchingdon, UK					
Influent dissolved phase					
0	0	9.1			
Influent particulate phase					
	0	0	2.3		
Sludge		205	321	432	
Effluent dissolved phase					
	0	0	0		
Effluent particulate phase					
	0	0	0		
Wickford, UK					
Influent dissolved phase					
	0	0	4.6		
Influent particulate phase					
	0	0	0		
Sludge		89.6	112	329	
Effluent dissolved phase					
	0	0	0		
Effluent particulate phase					
	0	0	0		
S. Woodham Ferrers, UK					
Influent dissolved phase					
0	0	0			
Influent particulate phase					
	0	0	0		
Sludge		233	547	798	
Effluent dissolved phase					
	0	0	0		
Effluent particulate phase					
	0	0	0		
Chelmsford, UK					
Influent dissolved phase					
	0	0	4.3		
Influent particulate phase					
	0	0	0		
Sludge		541	897	1245	
Effluent dissolved phase					
	0	0	0		
Effluent particulate phase					
	0	0	0		
STP1, Netherlands					
Influent	670	0	0	ng/L	
Sludge		0	0	0	ug/kg dw
Effluent	0	0	0	ng/L	
STP2, Netherlands					
Influent	3800	0	0		
Sludge		0	0	48	
Effluent	9	0	8.7		
STP3, Netherlands					
Influent	40	0	0		
Sludge		15	0	5.4	
Effluent	0 0		5.1		
STP4, Netherlands					
Influent	75	0	180		

Sludge		440	120	760
Effluent	0	0	0	
STP5, Netherlands				
Influent	0	0	0	
Sludge		-	-	-
Effluent	1.4	0	0	
STP6, Netherlands				
Sludge		7.6	0	20
STP7, Netherlands				
Sludge		3.5	0	3.6
STP8, Netherlands				
Sludge		7.4	0	13
STP9, Netherlands				
Sludge		0	0	0
STP10, Netherlands				
Sludge		0	0	0
Portlaoise, Ireland				
Sludge		280	349	604
Sludge		372	523	750
Clonmel, Ireland				
Sludge		3.9	0	149
Sludge		10.1	0.13	258
Cork, Ireland				
Sludge		2300	1800	3410

Population equivalents:
STP 1-4, 7 and 10 high treatment capacity, 200 000 - 750 000
STP 5, 6 and 8 100 000 - 150 000
UK varying from 4 750, Latchingdon, to 143 000, Chelmsford.

D. SEDIMENT (ug/kg ww)

Scheldt Basin (Data from (de Boer et al., 2002) as reported in draft EU RA)

	A	B		G		
Warmebeek Achel-kluis				0	0	0
Moervaart Daknam		0		0	0	
Benede Nete Duffel		0		0	0.5	
Grote Beverdijk Lo-R.		0		0	0	
Ijzer Nieuwpoort		0		0	0.9	
Durne Lokeren			0	0	0	
Leie Wervik	0	0		0		
Leie Wevelgem			0	0	0	
Leie Oselgem	0	0		0		
Leie St Martens		7		0	31	
Scheldt Doel	0	0		0		
Scheldt Grens	0	0		82		
Scheldt Oudenaarde		180		60	710	
Antwerp Kruisschansbr.				0	0	0
Scheldt Kastelt		0		0	5	
Scheldt Kennedyt.		0.3		0	0	
Dender Appels			0.3		0	0
Dender Ninovet			0	0	0.2	

Dutch Rivers (Data from (de Boer et al., 2002) as reported in draft EU RA)

Waal Tiel	0	0		0		
Rhine Lobith	0	4		4		
Hollans Diep	0	0		10		
Haringvliet West		0		0	6	
Haringvliet East		0		0	0	
Nieuwe Mervede			2	0	1	
Meuse Eijsden			0	0	0	

Meuse Keizersveer	3	0	0	
Roer Vlodrop	0	0	0	
UK	42	16-980	12-550	ppb dw
Ireland	0.3	0	0-30	
Dublin Bay, Ire	0	0-1	0-11	
	0	0	0	
Norway		0-9	0-4	0-79 ng/kg ww

E. Landfill LEACHATE (Data from draft EU Risk Assessment)

UK	0	0	0	ng/L (dissolved)
UK	0	0	0	ng/L (particulate)
Netherlands				
	0-7000	0-13	0-36000	ug/kg dw
Ireland	0	0	0	ng/L (dissolved)
Ireland	0	0	0	ng/L (particulate)

28.12.2004

28.12.2004

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media : other: 1000 kg/hr to air, water, and soil
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: EPIwin, Level III Fugacity Model
Year : 2004

Attached document : HBCD's partitioning in the environment was estimated using the Level III Fugacity Model of EPIwin, V3.04. At default emissions of 1000 kg/hr to each of air, water and soil, HBCD was predicted to dsitribute primarily to soil (40.1%) and sediment (57.9%). Only 2.06% was predicated to partition to water with neglibilbe levels to air (0.000685%). The soil Koc was calculated by the model to be 2.25 x 10+7. The percent reacted in air, water, soil and sediment was estimated as 0.304, 3.26, 63.3 and 22.9%, respectively. The percent advected in air, water, soil and sediment was estimated as 0.0225, 6.77, 0, 3.8%, repectively. The overall half-lives (hr) estimated (based on Biowin (Ultimate) and Aopwin) in air, water, soil and sediment were 51.19, 1440, 1440 and 5760, respectively.

The model was also run with emissions solely to either air, water or soil. If released solely to air, HBCD was predicted to partition to soil (77.7%) and sediment (21.6%). It was expected to be 97.1% reacted in soil and sediment with only 2.91% undergoing advection. If released soley to

water, HBCD was predicted to partition to sediment (96.6% with 3.44% to water. The percent reacted would be 71.2% adn 28.8% advected. If released solely to soil, HBCD was predicted to partition 99.9% to soil with 100% reaction.

27.12.2004

3.3.2 DISTRIBUTION

27.12.2004

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge
Concentration	:	15 mg/l related to Test substance related to
Contact time	:	3 hour(s)
Degradation	:	(±) % after
Result	:	
Deg. product	:	
Method	:	other: OECD 209
Year	:	2003
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : Activated Sludge Respiration Inhibition Test
(Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD (209) guidelines and Good Laboratory Practices.

HBCD's effect on the respiration of activated sludge microorganisms was assessed using control, reference and treatment groups. The control group was used to determine the background respiration rate of the sludge and was not dosed with the test or reference substance. The reference group was dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at nominal concentrations of 3, 15 and 50 mg/L. The test substance was dosed at a limit concentration of 15 mg/L. After an exposure period of ~ three hours, the respiration rates of the test solutions were measured using a dissolved oxygen meter. The individual respiration rates of the two controls were 60.5 and 55.5 mg O2/L/hr. The difference between the two control respiration rates was 9.0% and was within the 15% difference limit established for the test. The validity of the test was further supported by the results from the 3,5-dichlorophenol reference group, which resulted in an EC50 of 5.2 mg/L and was within the 5 to 30 mg/L range considered acceptable for the test. An average of 29.1 percent inhibition was observed in the treatment group.

Schaefer E and Siddiqui A. 2003. Hexabromocylcododecane (HBCD): An Activated Sludge, Respiration Inhibition Test. Project Number: 439E-108A. Wildlife International, Ltd. Easton, MD.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint
29.12.2004 (6)

Type : aerobic
Inoculum : activated sludge, domestic
Concentration : 7.7 mg/l related to Test substance
related to
Contact time : 28 day(s)
Degradation : = 0 (±) % after 28 day(s)
Result : under test conditions no biodegradation observed
Deg. product : no
Method : EPA OTS 796.3200
Year : 1996
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : Closed Bottle Test For Biodegradability (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to OECD 301D, OPPTS 3200, and Good Laboratory Practices.

HBCD was tested for ready biodegradation in a 28 day closed bottle test at a concentration of 7.7 mg/L by measuring dissolved oxygen uptake and expressing it as a percentage of the theoretical oxygen demand or chemical oxygen demand. No biodegradation was observed; the percent biodegradation was 0.

Schaefer, E and Haberlein, D., 1996, Hexabromocyclododecane (HBCD): Closed Bottle Test. Project No.: 439E-102. Wildlife International Ltd. Easton, MD.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.12.2004 (7)

Type : aerobic
Inoculum : other: aerobic water/sediment microcosms
Contact time : 119 day(s)
Degradation : >= 90 (±) % after 21 day(s)
Result : other: half-lives in 2 river sediments = 11 and 32 days in aerobic microcosms.
Deg. product : not measured
Method : other: OECD 308
Year : 2003
GLP : yes
Test substance : other TS

Attached document : Transformation in Aerobic and Anaerobic Water/Sediment Microcosms (Sponsor: ACC Brominated Flame Retardant Industry Panel and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. Its composition was 8.68% alpha, 6.12% beta, and 85.19% gamma. This study was performed according to Good Laboratory Practices and OECD Guideline 308.

The transformation of hexabromocyclododecane (HBCD) was determined in aerobic and anaerobic water/sediment microcosms based on the Organization for Economic Co-Operation and Development (OECD) Test

Guideline 308 "Aerobic and Anaerobic Transformation in Aquatic Sediment Systems." Laboratory batch microcosms were prepared with authentic water and sediment collected from two rivers in the eastern United States. Aerobic microcosms were pre-incubated at 20 ± 1 °C for 49 days and maintained by periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen. Anaerobic microcosms were prepared in an anoxic atmosphere (70% N2, 28% CO2, and 2% H2). The microcosms were pre-incubated at 23 ± 1 °C for 43 to 44 days to allow the microcosms to stabilize. HBCD was then added to the microcosms at nominal concentrations ranging from 34 to 89 ng/g (sediment dry weight). Biologically inhibited (i.e., abiotic) controls were prepared by steam sterilization of the sediment/water mixture prior to the addition of HBCD. Microcosms were incubated in the dark at 20 ± 1 °C for 119 days. The concentration of HBCD in the microcosms was determined at selected time intervals in the water and sediment phases utilizing high performance liquid chromatography-mass spectrometry (LC-MS). Aerobic microcosms were analyzed on days 0, 1, 7, 21, 64, 91, and 119, while anaerobic microcosms were analyzed on days 0, 1, 7, 14, 61(or 62), 91, and 119.

HBCD concentrations decreased over time in both the aerobic and anaerobic microcosms.

HBCD concentrations in the viable aerobic microcosms from both river systems decreased at least 90% within 21 days, while the corresponding decreases in the abiotic controls ranged from 7 to 62%. Disappearance of HBCD was observed in both the viable and abiotic anaerobic microcosms with the rate of loss more rapid in the viable microcosms, with HBCD reaching non-detected levels within 7 days. In contrast, HBCD concentrations in the abiotic controls decreased from 48 to 62% after 14 days. Pseudo-first order kinetics rate constants for the biotransformation of HBCD were determined by subtracting the abiotic rate constant from the viable rate constant. Biotransformation half-lives for HBCD in the two river systems were determined to be 11 and 32 days in the aerobic microcosms and 1.1 and 1.5 days in the anaerobic microcosms. Brominated degradation products were not detected in the sediment and water layers or in the headspace of the microcosms.

The purpose of this study was to determine the environmental lifetime of HBCD under realistic environmental conditions. Laboratory microcosms were used to evaluate the transformation of HBCD in aerobic and anaerobic water/sediment microcosms. Sediment degradation processes are expected to play a major role in determining the environmental lifetime of HBCD since, upon release into the environment, the majority of HBCD is likely to partition into the soil or sediment compartments. In this study, HBCD loss was observed in both viable and abiotic sediments although the rates were appreciable faster in the viable sediments. Biologically mediated transformation processes (i.e., biotransformation) accelerated the rate of loss of HBCD when compared to the biologically inhibited (i.e., heat-treated) microcosms. Brominated degradation products were not detected in any of the sediment microcosms.

Over the last several years a number of international protocols (EC 1996, UNECE 1998, UNEP 2000) have been put forth for the classification of chemicals as persistent (P), bioaccumulative (B), and toxic (T). The criteria for persistence in these initiatives includes half-lives in soil and sediments ranging from 120 to 180 days. In this investigation the resulting biotransformation half-lives for HBCD in the two river systems were determined to be 11 and 32 days in the aerobic sediments and 1.1 and 1.5 days in the anaerobic sediments, respectively.

Limited information is available for the reactions of HBCD in the

environment. However, the aerobic degradation and mineralization of a similar type of cyclic aliphatic halogenated fire retardant, FR-651A (mixture of pentabromo-chlorocyclohexane, tetrabromo-dichlorocyclohexane, and tribromotrichlorocyclohexane) has been observed. A soil half-life of ~11 days, based upon disappearance of 14C-FR-651A from soil, was reported. Complete degradation of 14C-FR-651A was also observed with a mineralization half-life on the order of 93 days.

An examination of the reactions of brominated aliphatic compounds that contain structural features similar to HBCD can also provide insight into the possible reaction pathways available for HBCD. Brominated aliphatic compounds are known to be susceptible to both hydrolytic and nuclelophilic attack. The reactivity of halogenated aliphatic compounds depends of the strength of the carbon-halogen bond, and increases in the order of F < Cl < Br. For example, the hydrolysis half-lives at pH 7 and 25 °C for methyl fluoride, methyl chloride, and methyl bromide are 1.1 x 104, 340, and 20 days, respectively. At environmental pH's neutral and base catalyzed hydrolysis reactions are most important, and depend on the degree and patterns of halogen substitution. Sulfur based nucleophiles can react rapidly with halogenated aliphatic compounds, thereby reducing their lifetimes in the environment. The half-life of 1-bromohexane in water at 25 °C was reduced from 20 days to approximately one day in 5 mM HS- or 0.07 mM polysulfide (Sx2-). Similarly, the half-life of 1,2-dibromoethane in water at 25°C was reduced from 1,000 days to 4 days in the presence of 5 mM HS-. Sulfide and polysulfides would be expected to be present in sediments at low redox potentials. Thus, reaction of HBCD with these sulfur-based nucleophiles may partly explain the loss of HBCD in the microcosm studies.

Halogenated aliphatic compounds are susceptible to reductive dehalogenation reactions in natural reducing environments. The rates of reactions vary depending on the strength and electron affinity of the carbon-halogen bond, and the stability of the carbon-radical species resulting from the initial electron transfer that occurs. Vicinal dehalogenation reactions are particularly important, and are dependent on the halogen atom. Half-lives for vicinal dehalogenation of 1,2-diiodoethane, 1,2-dibromoethane, and 1,2-dichloroethane are 0.6, 55, and 1.8 x 104 hours, respectively. The rapid disappearance of HBCD in the anaerobic sediment microcosms may be partly explained by reductive dehalogenation reactions. In addition, the disappearance of HBCD in the aerobic sediment microcosms may also be at least partly explained by reductive dehalogenation reactions. Anaerobic gradients often occur below the surface of sediments that are exposed to an aerobic water column. Such gradients would be expected to form in the static microcosms used in this study.

Based upon these results, HBCD is not persistent. Its half-lives in sediment are below the criteria specified by the various international protocols and specifically below the 120 days value specified in the European Commission's Technical Guidance Document on Risk Assessment (EC 1996).

Davis J, Gonsior S and Marty G. Evaluation Of Aerobic And Anaerobic Transformation Of Hexabromocyclododecane In Aquatic Sediment Systems. Study Number 021081. Environmental Chemistry Research Laboratory, Toxicology & Environmental Research and Consulting. The Dow Chemical Company Midland, Michigan. 2003.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.01.2005 (8)

Type	:	anaerobic
Inoculum	:	other: water/sediment microcosms
Contact time	:	119 day(s)
Degradation	:	= 100 (±) % after 7 day(s)
Result	:	other: half-lives in 2 river sediments = 1.1 and 1.5 days in the anaerobic microcosms.
Deg. product	:	not measured
Method	:	other: OECD 308
Year	:	2003
GLP	:	yes
Test substance	:	other TS

Attached document : Transformation in Aerobic and Anaerobic Water/Sediment Microcosms (Sponsor: ACC Brominated Flame Retardant Industry Panel and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. Its composition was 8.68% alpha isomer, 6.12% beta isomer and 85.19% gamma isomer. This study was performed according to Good Laboratory Practices and OECD Guideline 308.

The transformation of hexabromocyclododecane (HBCD) was determined in aerobic and
anaerobic water/sediment microcosms based on the Organization for Economic Co-Operation and Development (OECD) Test Guideline 308 "Aerobic and Anaerobic Transformation in Aquatic Sediment Systems." Laboratory batch microcosms were prepared with authentic water and sediment collected from two rivers in the eastern United States. Aerobic microcosms were pre-incubated at 20 ± 1 °C for 49 days and maintained by periodically exchanging the headspace of the microcosms with ambient air
to replenish oxygen. Anaerobic microcosms were prepared in an anoxic atmosphere (70% N2, 28% CO2, and 2% H2). The microcosms were pre-incubated at 23 ± 1 °C for 43 to 44 days to allow the microcosms to stabilize. HBCD was then added to the microcosms at nominal concentrations ranging from 34 to 89 ng/g (sediment dry weight). Biologically inhibited (i.e., abiotic) controls were prepared by steam sterilization of the sediment/water mixture prior to the addition of HBCD. Microcosms were incubated in the dark at 20 ± 1 °C for 119 days. The concentration of HBCD in the microcosms was determined at selected time intervals in the water and sediment phases utilizing high performance liquid chromatography-mass spectrometry (LC-MS). Aerobic microcosms were analyzed on days 0, 1, 7, 21, 64, 91, and 119, while anaerobic microcosms were analyzed on days 0, 1, 7, 14, 61(or 62), 91, and 119.

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or in the headspace of the microcosms.

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Over the last several years a number of international protocols (EC 1996, UNECE 1998, UNEP 2000) have been put forth for the classification of chemicals as persistent (P), bioaccumulative (B), and toxic (T). The criteria for persistence in these initiatives includes half-lives in soil and sediments ranging from 120 to 180 days. In this investigation the resulting biotransformation half-lives for HBCD in the two river systems were determined to be 11 and 32 days in the aerobic sediments and 1.1 and 1.5 days in the anaerobic sediments, respectively.

Limited information is available for the reactions of HBCD in the environment. However, the aerobic degradation and mineralization of a similar type of cyclic aliphatic halogenated fire retardant, FR-651A (mixture of pentabromo-chlorocyclohexane, tetrabromo-dichlorocyclohexane, and tribromotrichlorocyclohexane) has been observed. A soil half-life of ~11 days, based upon disappearance of 14C-FR-651A from soil, was reported. Complete degradation of 14C-FR-651A was also observed with a mineralization half-life on the order of 93 days.

An examination of the reactions of brominated aliphatic compounds that contain structural features similar to HBCD can also provide insight into the possible reaction pathways available for HBCD. Brominated aliphatic compounds are known to be susceptible to both hydrolytic and nuclelophilic attack. The reactivity of halogenated aliphatic compounds depends of the strength of the carbon-halogen bond, and increases in the order of F < Cl < Br. For example, the hydrolysis half-lives at pH 7 and 25 °C for methyl fluoride, methyl chloride, and methyl bromide are 1.1 x 104, 340, and 20 days, respectively. At environmental pH's neutral and base catalyzed hydrolysis reactions are most important, and depend on the degree and patterns of halogen substitution. Sulfur based nucleophiles can react rapidly with halogenated aliphatic compounds, thereby reducing their lifetimes in the environment. The half-life of 1-bromohexane in water at 25 °C was reduced from 20 days to approximately one day in 5 mM HS- or 0.07 mM polysulfide (Sx2-). Similarly, the half-life of 1,2-dibromoethane in water at 25°C was reduced from 1,000 days to 4 days in the presence of 5 mM HS-. Sulfide and polysulfides would be expected to be present in sediments at low redox potentials. Thus, reaction of HBCD with these sulfur-based nucleophiles may partly explain the loss of HBCD in the microcosm studies.

Halogenated aliphatic compounds are susceptible to reductive dehalogenation reactions in natural reducing environments. The rates of reactions vary depending on the strength and electron affinity of the carbon-halogen bond, and the stability of the carbon-radical species resulting from the initial electron transfer that occurs. Vicinal dehalogenation reactions are particularly important, and are dependent on

the halogen atom. Half-lives for vicinal dehalogenation of 1,2-diiodoethane, 1,2-dibromoethane, and 1,2-dichloroethane are 0.6, 55, and 1.8 x 104 hours, respectively. The rapid disappearance of HBCD in the anaerobic sediment microcosms may be partly explained by reductive dehalogenation reactions. In addition, the disappearance of HBCD in the aerobic sediment microcosms may also be at least partly explained by reductive dehalogenation reactions. Anaerobic gradients often occur below the surface of sediments that are exposed to an aerobic water column. Such gradients would be expected to form in the static microcosms used in this study.

Based upon these results, HBCD is not persistent. Its half-lives in sediment are below the criteria specified by the various international protocols and specifically below the 120 days value specified in the European Commission's Technical Guidance Document on Risk Assessment (EC 1996).

Davis J, Gonsior S and Marty G. Evaluation Of Aerobic And Anaerobic Transformation Of Hexabromocyclododecane In Aquatic Sediment Systems. Study Number 021081. Environmental Chemistry Research Laboratory, Toxicology & Environmental Research and Consulting. The Dow Chemical Company Midland, Michigan. 2003.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.01.2005 (8)

Type : aerobic
Inoculum : other: soil microcosms
Contact time : 119 day(s)
Degradation : = 75 (±) % after 119 day(s)
Result : other: half-life ~ 63 days
Deg. product : not measured
Method : other: OECD 308
Year : 2003
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : Transformation in Aerobic and Anaerobic Soil Microcosms (BFRIP and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to Good Laboratory Practices.

The transformation of HBCD was determined in aerobic and anaerobic soils based on the Organization for Economic Co-Operation and Development (OECD) Test Guideline 307 "Aerobic and Anaerobic Transformation in Soil." Soil microcosms were prepared by adding a sandy loam surface soil to serum bottles sealed with Teflon Ò coated septa. Aerobic microcosms were prepared by adjusting the soil moisture to 20% (by weight) and periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen. The microcosms were pre-incubated at 20 ± 1 °C for 35 days. Anaerobic microcosms were prepared in an anaerobic atmosphere (70% N2, 28% CO2, and 2% H2) by flooding the soil with water and pre-incubating the microcosms at 23 ± 1 °C for 43 days to allow low redox (e.g., methanogenic) conditions to develop. HBCD was then added to microcosms at a nominal concentration of 25 ng/g (soil dry weight), together with activated sludge (5 mg/g, dry weight basis) from a municipal wastewater treatment plant to simulate sludge land treatment applications. Biologically inhibited (abiotic) controls were prepared by

steam sterilization prior to the addition of HBCD. Microcosms were incubated in the dark at 20 ± 1 °C for 119 days. The concentration of HBCD in the microcosms was determined at selected time intervals utilizing high performance liquid chromatography-mass spectrometry (LC-MS). Aerobic microcosms were analyzed on days 0, 1, 7, 21, 48, 65, and 119, while anaerobic microcosms were analyzed on days 0, 1, 7, 21, 56, 91, and 119.

HBCD concentrations decreased over time in both the aerobic and anaerobic soils. HBCD concentrations decreased 75% over 119 days in the viable aerobic soil microcosms, compared to a 3% decrease in the abiotic controls, indicating that biological processes were responsible for most of the losses observed. Under anaerobic conditions, HBCD concentrations decreased 92% over 21 days in the viable microcosms compared to a less than 1% decrease in the abiotic controls. Pseudo-first order kinetics rate constants for the biotransformation of HBCD were determined by subtracting the abiotic rate constant from the viable rate constant. Biotransformation half-lives for HBCD were determined to be 63 and 6.9 days in the aerobic and anaerobic soils, respectively. Brominated degradation products were not detected in the soil or in the headspace of the microcosms.

The purpose of this study was to determine the environmental lifetime of HBCD under realistic environmental conditions. Laboratory microcosms were used to evaluate the transformation of HBCD in aerobic and anaerobic soils. Soil degradation processes are expected to play a major role in determining the environmental lifetime of HBCD since, upon release into the environment, the majority of HBCD is likely to partition into the soil or sediment compartments. In this study, HBCD loss was observed in both viable and abiotic soil microcosms although the rates were appreciably faster in the viable soils. Biologically mediated transformation processes (i.e. biotransformation) accelerated the reate of loss of HBCD when compared to the biologically inhibited (ie. heat-treated) soils. No bromianted degradaiton products were observed in either system.

Over the last several years a number of international protocols (EC 1996, UNECE 1998, UNEP 2000) have been put forth for the classification of chemicals as persistent (P), bioaccumulative (B), and toxic (T). The criteria for persistence in these initiatives includes half-lives in soil and sediments ranging from 120 to 180 days. In this investigation the resulting biotransformation half-lives for HBCD were determined to be 63 and 6.9 days in the aerobic and anaerobic soils, respectively. Based upon these results HBCD is not persistent. The half-lives in soil are below the criteria specified by the various international protocols.

Limited information is available for the reactions of HBCD in the environment. However, the aerobic degradation and mineralization of a similar type of cyclic, aliphatic halogenated fire retardant, FR-651A (mixture of pentabromochlorocyclohexane, tetrabromo-dichlorocyclohexane, and tribromotrichlorocyclohexane) was observed. A soil half-life of ~11 days based upon disappearance of 14 C-FR-651A from soil was reported. Complete degradation of 14C-FR-651A was also observed with mineralization half-life on the order of 93 days.

An examination of the reactions of brominated aliphatic compounds that contain structural features similar to HBCD can also provide insight into the possible reaction pathways available for HBCD. Brominated aliphatic compounds are known to be susceptible to both hydrolytic and nuclelophilic attack. The reactivity of halogenated aliphatic compounds increases in the order of F < Cl < Br. For example, the hydrolysis half-lives at pH 7 and 25 °C for methyl fluoride, methyl chloride, and methyl bromide are 1.1 x 104, 340, and 20 days, respectively. At environmental pH's, neutral and base

catalyzed hydrolysis reactions are most important, and depend on the degree and patterns of halogen substitution.

Sulfur based nucleophiles can react rapidly with halogenated aliphatic compounds, thereby reducing their lifetimes in the environment. The half-life of 1-bromohexane in water at 25 °C was reduced from 20 days to approximately one day in 5 mM HS - or 0.07
mM polysulfide (Sx 2-). Similarly, the half-life of 1,2-dibromoethane in water at 25 °C was reduced from 1,000 days to 4 days in the presence of 5 mM HS-. Sulfide and polysulfides would be expected to be present in soils at low redox potentials. Thus, reaction of HBCD with these sulfur-based nucleophiles may partly explain the loss of HBCD in the soil studies.

Since HBCD contains three pairs of vicinal bromine atoms, the transformation of simple aliphatic compounds containing vicinal bromine atoms may provide insight into possible reaction pathways for HBCD. For example, 1,2-dibromoethane reacts with nucleophiles via both substitution and elimination reactions. Reaction with HS- via an "SN2" substitution reaction results in the formation of HS-CH2-CH2-SH, while an elimination reaction under alkaline conditions results in the formation of H2C=CHBr. A combination of elimination and substitution reactions can result in the formation of a mixture of HO-H2-CH2-OH and H2C=CHBr. Similar mechanisms may be responsible for the loss of HBCD observed in this study.

Halogenated aliphatic compounds are susceptible to reductive dehalogenation reactions in natural reducing environments. The rates of reactions vary depending on the strength and electron affinity of the carbon-halogen bond and the stability of the carbon-radical species resulting from the initial electron transfer that occurs. Vicinal dehalogenation reactions are particularly important, and are dependent on the halogen atom. Half-lives for vicinal dehalogenation of 1,2-diiodoethane, 1,2-dibromoethane, and 1,2-dichloroethane are 0.6, 55, and 1.8 x 104 hours, respectively. The rapid disappearance of HBCD in the anaerobic soil microcosms may be partly explained by reductive dehalogenation reactions.

Based upon these results, HBCD is not persistent. Its half-lives in soil are clearly below the criteria for persistence specified in various international protocols (UNECE 1966, UNEP 2001).

Davis J, Gonsior S and Marty G. 2003. Evaluation Of Aerobic And Anaerobic Transformation Of Hexabromocyclododecane In Soil. Study Number 021082. Environmental Chemistry Research Laboratory. Toxicology & Environmental Research and Consulting. The Dow Chemical Company. Midland, MI.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.01.2005 (5)

Type : anaerobic
Inoculum : other: soil microcosms
Contact time : 119 day(s)
Degradation : = 92 (±) % after 21 day(s)
Result : other: half-life ~6.9 days
Deg. product : not measured
Method : other: OECD 307
Year : 2003
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : Transformation in Aerobic and Anaerobic Soil Microcosms (Sponosr: ACC

BFRIP and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to Good Laboratory Practices.

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ranging from 120 to 180 days. In this investigation the resulting biotransformation half-lives for HBCD were determined to be 63 and 6.9 days in the aerobic and anaerobic soils, respectively. Based upon these results HBCD is not persistent. The half-lives in soil are below the criteria specified by the various international protocols.

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Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.01.2005 (5)

Type :
Inoculum : other:activated and digester sludge, river sediment, and surface soil
Contact time :
Degradation : (±) % after
Result : other: degradation of all isomers in anaerobic digester sludge and in freshwater aerobic and anaerobic sediment microcosms
Deg. product : yes
Method : other:OECD 302B; 307, 308; ISO 11734
Year : 2004
GLP : yes
Test substance : other TS: [14C]HBCD
Deg. products : dibromocyclododecadiene
tetrabromocyclododecene
27070-59-3 248-206-1 cyclododecatriene

Attached document : Investigation of the biodegradation of [14C]Hexabromocyclododecane in sludge, sediment, and soil (European Brominated Flame Retardant Industry Panel)

This study investigated the biodegradation of the three HBCD stereoisomers, alpha, beta and gamma, and the identity of major degradation products. The formation and identification of degradation products of HBCD was assessed in activated and digester sludge, river sediment, and surface soil. Both aerobic and anaerobic biodegradation were evaluated in laboratory reaction mixtures and batch microcosms. This study was performed according to the relevant OECD Guidelines (302B; 307, 308), ISO 11734, and Good Laboratory Practices.

To generate sufficient levels of [14C]degradation products for their identification, [14C]HBCD was added to reaction mixtures and microcosms at nominal concentrations ranging from 3 to 5 mg/kg (or mg/L), exceeding the water solubility of the test material by greater than an order or magnitude. Duration of the studies ranged from approximately 60 to 112 days. Reaction mixtures and batch microcosms were extracted and analyzed by high performance liquid chromatography (HPLC) with radiochemical detection to follow the degradation of the 3 stereoisomers and formation of [14C]products. Product identification was facilitated by analyses of extracts from the soil, sediment and sludge mixtures by HPLC-atmospheric pressure photo ionization-mass spectrometry (APPI-MS) or gas chromatography-electron impact ionization-mass spectrometry (GC-EI-MS).

Substantial biological transformation of [14C]HBCD was observed in the anaerobic digester sludge and in freshwater aerobic and anaerobic sediment microcosms. Conversely, no degradation of HBCD was noted in the soil microcosms incubated under aerobic conditions. In the digester sludge and sediment, degradation of each of the three stereoisomers occurred over the course of the study. Little difference was noted in the disappearance of the three stereoisomers, indicating similarity in the extent of degradation of each isomer.

Concomitant with the loss of [14C]HBCD in the sludge and sediment test mixtures was formation of three [14C]products. Using a combination of HPLC-APPI-MS and GC-EI-MS these metabolites were identified as tetrabromocyclododecene, dibromocyclododecadiene, and cyclododecatriene. These products suggest HBCD is sequentially debrominated via dihaloelimination by naturally occurring microorganisms in wastewater sludge and aquatic sediment. During each sequential debromination, two bromines are lost from vicinal carbons with the subsequent formation of a double bond between the adjacent carbon atoms. These results demonstrate microorganisms naturally occurring in aquatic sediment and wastewater sludges can completely debrominate HBCD.

Davis JW, Gonsior SJ, Markham DA, and Marty GT. 2004. Investigation of the biodegradation of [14C]hexabromocyclododecane in sludge, sediment, and soil. Laboratory Project Study ID 031178. Toxicology & Environmental Research and Consulting. The Dow Chemical Company, Midland, MI.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
11.01.2005 (9)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 34 day(s) at 25 °C
Concentration : 3.4 µg/l
BCF : = 8974
Elimination : yes
Method : EPA OPPTS 850.1730
Year : 2000
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : Flow Through Bioconcentration In Rainbow Trout (Oncorhynchus mykiss) (Sponsor: ACC BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

Nominal test concentrations were 0, 0.34, and 3.4 ug HBCD/L. These doses are equivalent to gamma HBCD's water solubility and one tenth of its water solubility. Mean measured (LC/MS with heated nebulizer operated in the selected ion monitoring mode) test concentrations were 0, 0.18, and 1.8 ug HBCD/L. The length of the test was 70 days (35-day uptake, 35-day depuration). The steady bioconcentration factor (BCF) at a nominal concentration of 3.4 ug HBCD/L (1.8 ug HBCD/L measured) in whole fish was 8,974. This BCF was further defined as 4,650 in edible tissue and 12,866 in non-edible tissue. Steady state was not achieved at the nominal concentration of 0.34 ug HBCD/L due to an unexpected increase in tissue concentrations at day 35. The unexpected increase in tissue concentrations on day 35 may have been due to the variability in the measured water concentrations in this treatment group. The variability in turn is likely a function of the extremely low nominal concentration at this

dose level (0.34 ug HBCD/L). Thus, the calculated BCF in the nominal 3.4 ug HBCD/L treatment group is considered a better estimate than that in the 0.34 ug HBCD/L treatment group.

Drottar K. and Krueger H. 2000. Hexabromocyclododecane (HBCD): Flow-through bioconcentration test with rainbow trout (Oncorhynchus mykiss). Project No.: 439A-111. Wildlife International, Ltd. Easton, MD.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
28.12.2004 (10)

Species : other: Eisenia fetida
Exposure period : 28 day(s) at °C
Concentration :
BCF : = 4.5
Elimination :
Method :
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : Earthworm (Eisenia fetida) Survival and Reproduction

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

The artificial soil used in this study was characterized as sandy loam with an 80% sand, 8% silt, and 12% clay content. Nominal test concentrations were 0, 78.5, 157, 313, 625, 1,250, 2,500 and 5,000 mg HBCD/kg of dry soil. Mean measured concentrations at day 28 were <1.28 (Control), 61.2, 145, 244, 578, 1,150, 2,180, and 4,190 mg HBCD/kg of dry soil. Mean measured concentrations at day 56 were 56 Days: <1.35 (Control), 51.5, 128, 235, 543, 1,070, 2,020, and 3,990 mg HBCD/kg of dry soil. Measured tissue concentrations at day 28 were <0.200 (control), 3.40, 7.32, 16.8, 15.3, 53.0, 71.2, and 150 mg HBCD per gram of tissue.

No abnormal burrowing or avoidance behaviors were recorded during the first 60 minutes of testing.

Using the estimated soil Koc and the formula provided in the European Union's Technical Guidance Document, HBCD's calculated BCF in earthworms is:

BCFearthworm
= Cearthworm / Csoil
= Kearthworm-porewater x RHOsoil x 10+3 / Ksoil-water

=(0.15 mg/kg)(1.25 x 10+5)/4,190 mg/kg dry soil
= 4.5.

Thus, HBCD did not bioconcentrate in the earthworm.

Aufterheide et al. 2002. Effect of Hexabromocyclododecane on the Survival and Reproduction of the Earthworm, Eisenia fetida. ABC Study No. 47222. ABC Laboratories, Inc., Columbia, Missouri; Wildlife International, Inc., Easton, MD.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.12.2004 (11)

27.12.2004

3.8 ADDITIONAL REMARKS

Memo : Summary: HBCD Half-lives in Sediment and Soil

Attached document : Substances which are persistent is soil or sediments are typically defined as those wiht half-lives ranging from 120 to 180 days. HBCD's half-lives in aerobic and anaerobic soils were 63 and 6.9 days, respectively. In sediments collected from two river systems, HBCD's half-lives were 11 and 32 days in the aerobic water/sediment microcosms and 1.1 and 1.5 days in the anaerobic water/sediment microcosms.

Based upon these results, HBCD is not persistent. Its half-lives in soil and sediment are below the criteria specified by various international protocols (UNECE 1966, UNEP 2001, EU Technical Guidance Document 1996).

12.01.2005

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	µg/l
NOEC	:	>= 6.8 measured/nominal
Limit test	:	no
Analytical monitoring	:	yes
Method	:	EPA OPPTS 850.1075
Year	:	1997
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : 96-Hour Acute Toxicity Test With Rainbow Trout (Oncorhynchus mykiss) (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices.

HBCD was not acutely toxic to rainbow trout at the limit of the gamma stereoisomer's solubility. HBCD's 96 hour LC50, no mortality concentration and no observed effect concentration were all > than the gamma stereoisomer's water solubility. The highest nominal dose tested was twice that water solubility. Nominal test concentrations were 0, 1.5, 2.2, 3.2, 4.6 and 6.8 ug/L and corresponded to mean measured concentrations (HPLC with UV/VIS detector) of 0, 0.75, 1.5, 2.3, 2.3 and 2.5 ug/L, respectively.

Graves, W and Swigert, J. (1997) Hexabromocyclododecane (HBCD): A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (Oncorhynchus mykiss). Project Number: 439A-101. Wildlife International LTD, Easton, MD.

Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

29.12.2004 (12)

Type	:	flow through
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	88 day(s)
Unit	:	µg/l
NOEC	:	= 6.8 measured/nominal
Limit test	:	no
Analytical monitoring	:	yes
Method	:	OECD Guide-line 210
Year	:	2001
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : Fish Early Life Stage In Rainbow Trout (Oncorhynchus mykiss) (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current OECD guidelines and Good Laboratory Practices.

Rainbow trout embryos were exposed to nominal HBCD water concentrations of 0.43, 0.85, 1.7, 3.4 and 6.8 ug/L. The top two doses

represent gamma HBCD's water solubility (3.4 ug/L) and two times gamma HBCD's water solubility (6.8 ug/L). A negative control and solvent control group were also included. Mean measured concentrations (LC/MS with heated nebulizer operated in the selective ion monitoring mode) were 0.25, 0.47, 0.83, 1.8 and 3.7 ug/L. This method was designed to monitor for all 3 HBCD diastereomers; however, the trace residues of the alpha and beta diastereomers were evident in the water samples were below the established limits of quantitation. Comparison of the chromatograms from study initiation through study termination showed that the relative distribution of the HBCD diastereomers remained constant during the definitive study, and the gamma diastereomer measured results were consistent throughout the study.

Hatching success, time to hatch, time for larvae to swim-up, and post-hatch growth and survival were evaluated during the 88-day test. Rainbow trout exposed to HBCD at mean measured concentrations up to 3.7 ug/L (nominal concentration = 6.8 ug/L or twice HBCD's water solubility) for a 27-day hatching period and 61 days post-hatch showed no effects on hatching success, time to swim-up, larval survival, fry survival or growth. Consequently, HBCD was not chronically toxic to rainbow trout at concentrations at or above its limit of solubility. The NOEC for this study was 3.7 ug/L or 6.8 ug/L nominal (twice gamma HBCD's water solubility). The low-effect-concentration (LOEC) and maximum acceptable toxicant concentration (MATC) could not be determined due to absence of toxicity, but were considered >3.7 ug/L or >6.8 ug/L nominal (> twice gamma HBCD's water solubility).

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
28.12.2004 (13)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	flow through
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	µg/l
NOEC	:	>= 3.2 measured/nominal
EC50	:	> 3.2 measured/nominal
Limit Test	:	no
Analytical monitoring	:	yes
Method	:	EPA OPPTS 850.1010
Year	:	1997
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : Acute Toxicity to Aquatic Invertebrates: 48-Hour Acute Toxicity Test With Daphnia magna (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices.

HBCD was not acutely toxic to Daphnia magna. HBCD's 48 hour EC50, no mortality/immobility concentration, and no observed effect concentration (6.8 ug/L nominal) in Daphnia magna were all > than gamma HBCD's water solubility (3.4 ug/L measured). The highest nominal dose tested was twice gamma HBCD's measured water solubility. Nominal test concentrations were 0, 1.5, 2.2, 3.2, 4.6 and 6.8 ug/L which corresponded

to mean measured concentrations (HPLC with UV/VIS detector) of 0, 2.4, 1.8, 2.1, 2.3 and 3.2 ug/L, respectively.

Graves W and Swigert J. (1997) Hexabromocyclododecane (HBCD): a 48-hour flow-through acute toxicity test with the cladoceren (Daphnia magna). Project Number: 439A-102. Wildlife International Ltd., Easton, MD.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
29.12.2004 (14)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : biomass
Exposure period : 96 hour(s)
Unit : µg/l
NOEC : >= 3.7 measured/nominal
LOEC : > 3.7 measured/nominal
EC10 : > 3.7 measured/nominal
EC50 : > 3.7 measured/nominal
Limit test : no
Analytical monitoring : yes
Method : EPA OPPTS 850.5400
Year : 1997
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : 96-Hour Acute Toxicity Test With The Freshwater Alga (Selenastrum capricornutum) (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices. This study was performed to complete the EU base set.

HBCD was not acutely toxic to Selenastrum capricornutum. HBCD's 96 hour EC10, EC50, EC90 and no observed effect concentration were all > than HBCD's water solubility based on the gamma isomer. The highest nominal dose tested was twice gamma HBCD's water solubility. Dose levels were 0, 1.5, 2.2, 3.12 4.6 and 6.8 ug/L (nominal). The mean measured concentration (HPLC with UV/VIS detector) at the 6.8 ug/L dose was 3.7 ug/L.

Roberts C. and Swigert J. Hexabromocyclododecane (HBCD): A 96-Hour Toxicity Test with the Freashwater Alga (Selenastrum capricornutum). Wildlife International Ltd. Project Number: 439A-103. June 3, 1997. Wildlife International Ltd., Easton, MD.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
29.12.2004 (15)

Species : Skeletonema costatum (Algae)
Endpoint : other:cell densities, biomass and growth rate
Exposure period : 72 hour(s)
Unit : µg/l
NOEC : < 41 measured/nominal
EC50 : > 41 measured/nominal
Limit test : yes

Analytical monitoring	:	yes
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	2004
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : HBCD: A 72-Hour toxicity test with the marine diatom (Skeletonema costatum)

The objective of this study was to determine the toxicity of water-soluble components of hexabromocyclododecane (HBCD) to the marine diatom Skeletonema costatum, using a saltwater algal media passed through a generator column saturated with HBCD.

This study was performed according to Good Laboratory Practices, ISO 10253:1995, OECD 201, and EU Directive 92/69/EEC, Method C.3. The test article was a composite of the commercial products of Albemarle Corporation, Dead Sea Bromine Group and Great Lakes Chemical Corporation. Composite = 7.67% alpha, 5.15% beta and 83.04% gamma.

The experimental design was developed based on prelminary work. The marine diatom was exposed to a single test concentration (produced via water generator column) of HBCD in salwater algal media, a negative control (also referred to as Control with Column) and a media control (also referred to as Control no Column) for 72 hours. The single test concentration was prepared using algal media passed through a comlumn generator packed with a solid support coate with HBCD to achieve the test concentraiton of HBCD in saltwater algal medium. Measured test concentrations were determined from samples of test medium collected from teh treament and each control group at the beginning of the test and at test termination.

At test initiation an inoculum of the algal cells was added to each test chamber to achieve a nominal concentratin of approximately 77,000 Skeletonema cells/ml. Samples were collected from each replicate test chmaber at approximately 24-hour intervals during the test to determine cell densities, which were subsequently used to caluculate areas under the growth curve (biomass) and growth rates. Cell densitites, biomass and growth rates were used to calculate percent inhibitoin vlaues relative to the control over the 72-hour exposure period. No-oberved-effect-concentrations (NOEC) were determined at 72 hours through statistical evaluation of the cell densities, biomass and growth rates, as well as examination of the concentration-response pattern.

The analytical method for the 3 HBCD stereoisomers in saltwater algal medium was based on methodology developed by Wildlife International LTD. It used an HPLC interfaced with a triple quadrupole LC mass spectromer operated in single-quadrupole selective ion monitoring mode. The LOQ was 0.5 ug a.i./L. The Day 0 and 3 arithmetic mean measured test concentrations for Gamma, Beta, Alpha and total HBCD was 1.61, 8.86, 30.5 and 41.0 ug a.i./L, respectively. Arithmetic mean measured test concentrations were used to calculate the NOEC values.

S. costatum were exposed to a single concentraion of 41.0 ug a.i./L of toal HBCD and evaluated for effects on cell density, area under the growth curve (biomass) and growth rate. After 72 of exposure, inhibition of cell density, biomass and growth rate in the treated group was 19, 21 and 7.3%, respectively, relative to control no column. Similarly, relative to the control with column, inhibition of cell density, biomass and growth rate was 31, 31 and 11%, respectively. The treated group was statistically different from both control groups ($p<0.05$).

Since there were effects observed, the 72 hour NOEC and EC50, based on cell density, biomass and growth rate were
< 41.0 ug/ a.i./L and > 41.0 ug a.i./L total HBCD, respectively. The total HBCD concentration was composed of 1.61 ug a.i./L gamma; 8.86 ug a.i./L beta and 30.5 ug a.i./L alpha isomers.

Desjardins D, MacGregor J, Krueger H. 2004. Hexabromocyclododecane (HBCD): a 72-hour toxicity test with the marine diatom (Skeletonema costatum). Final Report. Wildlife International LTD Project Number: 439A-125. Wildlife International LTD, Easton, MD.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
29.12.2004 (16)

Species : other algae
Endpoint : biomass
Exposure period :
Unit :
Method : other
Year : 1987
GLP : no data
Test substance : other TS

Attached document : Walsh et al. 1987 reported testing the effect of media and test chemicals on acute toxicity in marine algae. HBCD was tested in 3 species of marine algae in 6 different media, and was not toxic at the limits of the gamma isomer's water solubility. The EC50's are as follows:
Chlorella sp 96 hr EC50 > 1500 ug/L);
S. costatum 72 hr EC50 9.3-12 ug/L;
T. pseudonana 72 hr EC50 50-370 ug/L).

Walsh et al. 1987. Ecotoxicology and Environmental Safety, 14, 215-222.

Reliability : (2) valid with restrictions
29.12.2004 (17)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : other
Species : activated sludge, domestic
Exposure period : 3 hour(s)
Unit : mg/l
Analytical monitoring : yes
Method : OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year : 2003
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : Activated Sludge Respiration Inhibition Test
(Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD (209) guidelines and Good Laboratory Practices.

HBCD's effect on the respiration of activated sludge microorganisms was assessed using control, reference and treatment groups. The control group was used to determine the background respiration rate of the sludge and was not dosed with the test or reference substance. The reference group was dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at

nominal concentrations of 3, 15 and 50 mg/L. The test substance was dosed at a limit concentration of 15 mg/L. After an exposure period of ~ three hours, the respiration rates of the test solutions were measured using a dissolved oxygen meter.

The individual respiration rates of the two controls were 60.5 and 55.5 mg O2/L/hr. The difference between the two control respiration rates was 9.0% and was within the 15% difference limit established for the test. The validity of the test was further supported by the results from the 3,5-dichlorophenol reference group, which resulted in an EC50 of 5.2 mg/L and was within the 5 to 30 mg/L range considered acceptable for the test. An average of 29.1 percent inhibition was observed in the treatment group.

Schaefer E and Siddiqui A. 2003. Hexabromocylcododecane (HBCD): An Activated Sludge, Respiration Inhibition Test. Project Number: 439E-108A. Wildlife International, Ltd. Easton, MD.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
28.12.2004 (6)

4.5.1 CHRONIC TOXICITY TO FISH

Species	:	Oncorhynchus mykiss (Fish, fresh water)
Endpoint	:	other: Hatching success, time to hatch, time for larvae to swim-up, and post-hatch growth and survival
Exposure period	:	88 day(s)
Unit	:	µg/l
NOEC	:	>= 3.7 measured/nominal
LOEC	:	> 3.7 measured/nominal
Analytical monitoring	:	yes
Method	:	OECD Guide-line draft "Early Life Stage Test (ELS-Test)"
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : Fish Early Life Stage In Rainbow Trout (Oncorhynchus mykiss) (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current OECD guidelines and Good Laboratory Practices.

Rainbow trout embryos were exposed to nominal HBCD water concentrations of 0.43, 0.85, 1.7, 3.4 and 6.8 ug/L. The top two doses represent gamma HBCD's water solubility (3.4 ug/L) and two times gamma HBCD's water solubility (6.8 ug/L). A negative control and solvent control group were also included. Mean measured concentrations (LC/MS with heated nebulizer operated in the selective ion monitoring mode) were 0.25, 0.47, 0.83, 1.8 and 3.7 ug/L. This method was designed to monitor for all 3 HBCD diastereomers; however, the trace residues of the alpha and beta diastereomers evident in the water samples were below the established limits of quantitation. Comparison of the chromatograms from study initiation through study termination showed that the relative distribution of the HBCD diastereomers remained constant during the definitive study, and the gamma diastereomer measured results were consistent throughout the study.

Hatching success, time to hatch, time for larvae to swim-up, and post-hatch growth and survival were evaluated during the 88-day test. Rainbow trout

exposed to HBCD at mean measured concentrations up to 3.7 ug/L (nominal concentration = 6.8 ug/L or twice HBCD's water solubility) for a 27-day hatching period and 61 days post-hatch showed no effects on hatching success, time to swim-up, larval survival, fry survival or growth. Consequently, HBCD was not chronically toxic to rainbow trout at concentrations at or above its limit of solubility. The NOEC for this study was 3.7 ug/L or 6.8 ug/L nominal (twice gamma HBCD's water solubility). The low-effect-concentration (LOEC) and maximum acceptable toxicant concentration (MATC) could not be determined due to absence of toxicity, but were considered >3.7 ug/L or >6.8 ug/L nominal (> twice gamm HBCD's water solubility).

Drottar et al. 2001. Hexabromocyclododecane (HBCD): An early life-stage toxicity test with the rainbow trout (Onchorhynchus mykiss). Project No.: 439A-112. Wildlife International, Ltd. Easton, MD.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.12.2004 (10)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	Daphnia magna (Crustacea)
Endpoint	:	other: survival, reproduction and growth
Exposure period	:	21 day(s)
Unit	:	µg/l
NOEC	:	= 3.4 measured/nominal
LOEC	:	= 5.6 measured/nominal
Analytical monitoring	:	yes
Method	:	EPA OTS 797.1330
Year	:	1998
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : Daphnia magna Life Cycle (21 Day) (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

Nominal test concentrations were 0.85, 1.7, 3.4, 6.8 and 13.6 ug HBCD/L water; dose levels were based on gamma HBCD's water solubility, 3.4 ug/L. Measured test concentrations (LC/MS with negative ion atmospheric pressure ionization) were 0.87, 1.6, 3.1, 5.6 and 11 ug HBCD/L water (based on the gamma stereoisomer).

No statistically significant effects on survival, reproduction or growth of Daphnia magna were seen at HBCD concentrations < 3.1 ug/L (measured). Thus, HBCD's no effect concentration (NOEC), based on survival, reproduction and growth, to daphnia magna for 21 days was equivalent to HBCD's water solubility. The measured NOEC in this study was 3.1 ug/L and corresponded to a nominal HBCD concentration of 3.4 ug/L, e.g. gamma HBCD's water solubility. The lowest observed effect concentration (LOEC) and the maximum acceptable toxicant concentration (MATC) based on survival, growth and reproduction were greater than HBCD's water solubility. The LOEC, 5.6 ug/L, corresponded to nominal concentrations twice gamma HBCD's water solubility. The effect seen at this dose level was a reduction in length. Survival and reproduction at the 5.6 ug/L dose level were not affected. The MATC, 4.2 ug/L, was calculated

as the mean of the NOEC and the LOEC. The MATC was greater than gamma HBCD's water solubility.

Drottar K. and Krueger H. 1998. Hexabromocyclododecane (HBCD): Flow-through life-cycle toxicity test with the cladocerna (Daphnia magna). Project No.: 439A-108. Wildlife International, Ltd. Easton, MD.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.12.2004 (18)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

Species : Hyalella
Endpoint : other: survival and growth, 5% TOC
Exposure period : 28 other: days
Unit : mg/kg sediment dw
NOEC : = 1000 measured/nominal
LOEC : > 1000 measured/nominal
Method : other:ASTM E 1706-95b, OPPTS 850.1735
Year : 2003
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : Prolonged Sediment Study with Hyalella azteca, 5% TOC (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The definitive study was performed according to current ASTM, OECD and GLP guidelines.

Non-GLP exploratory range-finding studies were performed with three freshwater species associated with sediment: oligochaetes (Lumbriculus variegatus), chironomids (Chironomus riparius) and amphipods (Hyalella azteca). All three species were tested at 50, 100, 500, 1000 mg/Kg dry sediment in two different types sediments, one with a 2% organic carbon content and the other with a 5% organic carbon content. Based on the results of the range-finding studies, the amphipods were found to be the most sensitive species in both sediment types, with clear effects in the 500 mg/Kg treatment group. This reports the results of HBCD exposure in the 5% organic content sediment in Hyalella azteca. A similar study in 2% organic carbon was also performed.

Groups of amphipods were exposed to a geometric series of six test concentrations and a negative control for 28 days under flow-through test conditions. Eight replicate test compartments were maintained in each treatment and control group, with 10 amphipods in each test compartment, for a total of 80 amphipods per test concentration. Each test compartment contained a quantity of sediment and overlying water. Additional replicates were added in the control group, low and high treatment groups for analytical sampling of water and sediment. The "analytical" replicates sampled on Day 0 contained no amphipods, while amphipods were added at test initiation to the "analytical" replicates sampled on Day 7 and at test termination.

Nominal test concentrations were 31, 63, 125, 250, 500 and 1000 mg/Kg of sediment based on the dry weight of the sediment. The results of the study are based on the nominal test concentrations. Overlying water, pore water and sediment samples were collected and analyzed from the "analytical replicates" of the control group and the lowest and highest test

concentrations. The collection and analysis were done approximately ten minutes after the addition of test organisms to the test system on Day 0, on Day 7 and at the end of the test. Results of the analyses were used to confirm the lowest and highest test concentrations.

Analysis of HBCD concentrations in the sediment, pore water and overlying water samples collected during test confirmed that the test article tended to remain in sediment and not move into the pore water or overlying water. Concentrations in sediment at the highest dose level ranged from 78.2 - 122% of nominal, while the lowest dose level ranged from below the limit of quantitation (12.5 mg/kg) to 197% of nominal. All overlying water samples contained no detectable HBCD. Pore water sample from the low concentration were also below the limit of quantitation, but those from the highest dose level, 1000 mg/kg sediment, had HBCD concentrations in the low ppm range. These values were all well above HBCD's water solubility and were believed the result of small particles of HBCD being extracted out of the pore water, artificially inflating the reported values.

Observations of amphipods in individual replicates appeared normal, with some mortality in the 0, 31, 63, 125, 250 and 500 mg/kg groups. Fungal growth was noted in all replicates.

The mean number of amphipods in the negative control, 31, 63, 125, 250, 500 and 1000 mg/Kg treatment groups at test termination was 9.1, 8.6, 5.9, 6.1, 7.0, 8.5 and 9.1, respectively. The mean numbers of amphipods in the 63, 125 and 250 mg/Kg treatment groups were found to be statistically different ($p < 0.05$) from the negative control group. Survival in the 31, 500 and 1000 mg/Kg treatment groups was similar to the control group and any differences were not statistically significant ($p>0.05$). Since the percent reduction in the number of organisms present at test termination in comparison to the negative control group was less than 50% in all treatment groups, the 28-day EC50 value was estimated to be greater than 1000 mg/Kg of dry sediment, the highest concentration tested. The percent reduction from the control in the 63, 125, and 250 mg/Kg treatment groups was 35.2, 33.0, and 23.1%, respectively. The mortality in these groups was moderate and there were clearly no effects at the 500 and 1000 mg/Kg treatment levels. Therefore, the mortality observed in the middle test concentrations was not considered treatment related since there was no evidence of a concentration dependent response.

The average dry weight per amphipod in the negative control group was 0.19 mg. The average dry weight per amphipod in the 31, 63, 125, 250, 500 and 1000 mg/Kg treatment groups was 0.17, 0.26, 0.22, 0.20, 0.19 and 0.19 mg, respectively. The dry weights were not significantly different ($p>0.05$) from the negative control weights, and any differences were not concentration-dependent. Therefore, there were no apparent effects on growth (dry weight) observed at test termination.

The 28-day EC50 value for amphipods (Hyalella azteca) exposed to hexabromocyclododecane in sediment was >1000 mg/Kg dry weight of sediment, the highest nominal concentration tested. Determination of the lowest-observed-effect-concentration (LOEC) and the no-observed-effect-concentration (NOEC) was based on an evaluation of the survival and growth (dry weight) data. The most sensitive parameter in this study was survival. Based on the results of this study, the LOEC was >1000 mg/Kg dry weight of sediment and the NOEC was 1000 mg/Kg dry weight of sediment.

Thomas J et al. 2003. Hexabromocyclododecane (HBCD): A Prolonged Sediment Toxicity Test with Hyalella azteca Using Spiked Sediment with 5% Total Organic Carbon. Project Number: 439A-120. Wildlife International, Ltd. Easton, MD.

Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
28.12.2004		(19)

Species	:	Hyalella
Endpoint	:	other: survival and growth, 2% TOC
Exposure period	:	28 other: days
Unit	:	mg/kg sediment dw
NOEC	:	= 1000 measured/nominal
NOEC	:	> 1000 measured/nominal
Method	:	other: ASTM E 1706-95b, OPPTS 850.1735
Year	:	2003
GLP	:	
Test substance	:	

Attached document : Prolonged Sediment Study with Hyalella azteca, 2% TOC (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The definitive study was performed according to current EPA, OECD and GLP guidelines.

Non-GLP exploratory range-finding studies were performed with three freshwater species associated with sediment: oligochaetes (Lumbriculus variegatus), chironomids (Chironomus riparius) and amphipods (Hyalella azteca). All three species were tested at 50, 100, 500, 1000 mg/Kg dry sediment in two different types sediments, one with a 2% organic carbon content and the other with a 5% organic carbon content. Based on the results of the range-finding studies, the amphipods were found to be the most sensitive species in both sediment types, with clear effects in the 500 mg/Kg treatment group. This reports the results of HBCD exposure in the 5% organic content sediment in Hyalella azteca. A similar study in 5% organic carbon was also been performed.

Groups of amphipods were exposed to a geometric series of six test concentrations and a negative control for 28 days under flow-through test conditions. Eight replicate test compartments were maintained in each treatment and control group, with 10 amphipods in each test compartment, for a total of 80 amphipods per test concentration. Each test compartment contained a quantity of sediment and overlying water. Additional replicates were added in the control group, low and high treatment groups for analytical sampling of water and sediment. The "analytical" replicates sampled on Day 0 contained no amphipods, while amphipods were added at test initiation to the "analytical" replicates sampled on Day 7 and at test termination.

Nominal test concentrations were 31, 63, 125, 250, 500 and 1000 mg/Kg of sediment based on the dry weight of the sediment. The results of the study are based on the nominal test concentrations. Overlying water, pore water and sediment samples were collected and analyzed from the "analytical replicates" of the control group and the lowest and highest test concentrations. The collection and analysis were done approximately ten minutes after the addition of test organisms to the test system on Day 0, on Day 7 and at the end of the test. Results of the analyses were used to confirm the lowest and highest test concentrations.

Analysis of HBCD concentrations in the sediment, pore water and overlying water samples collected during test confirmed that the test article tended to remain in sediment and not move into the pore water or overlying water. Concentrations in sediment at the highest dose level ranged from 82.8 - 115% of nominal, while the lowest dose level ranged from 49.5 to 125% of

nominal. All overlying water samples contained no detectable HBCD. Pore water sample from the low concentration were also below the limit of quantitation, but those from the highest dose level, 1000 mg/kg sediment, had HBCD concentrations in the low ppm range. These values were all well above HBCD's water solubility and were believed the result of small particles of HBCD being extracted out of the pore water, artificially inflating the reported values.

Observations of amphipods in individual replicates appeared normal, with some mortality in the 0, 31 and 1000 mg/kg groups. Fungal growth was noted in all replicates.

The mean number of amphipods in the 0, 31, 63, 125, 250, 500 and 1000 mg/kg groups at test termination was 7.4, 5.9, 7.8, 5.4, 6.9, 7.3, and 5.8, respectively. No statistical difference between treated and control groups were detected. The average dry weight per amphipod in the 31, 63, 125, 250, 500 and 1000 mg/kg treatment groups was 0.10, 0.13, 0.14, 0.12, 0.17 and 0.14, respectively, and were not significantly different from control ($p>0.05$).

Based on survival and growth (dry weight) data, the 28-day EC50 was > 1000 mg/kg of dry sediment, the highest concentration tested. The LOEC was > 1000 mg/kg and the NOEC was 1000 mg/kg dry weight of sediment.

Thomas S, Krueger H and Kendall T. 2003. Hexabromocyclododecane (HBCD): A Prolonged Sediment Toxicity Test with Hyalella azteca Using Spiked Sediment with 2% Total Organic Carbon. Project Number: 439A-119B. Wildlife International, Ltd. Easton, MD.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
29.12.2004 (20)

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species : other terrestrial plant
Endpoint : other: seedling emergence and growth
Exposure period : 21 day(s)
Unit : mg/kg soil dw
NOEC : >= 5000 measured/nominal
Method : other: OPPTS 850.4100, 850.4225
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : Seediling Emergence and Growth in Six Plant Species
(Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to EPA OPPTS 850.4100 and 850.4225; OECD Proposed for Revision of Guidleine 298; EPA/OECD Good Laboratory Practices.

The purpose of the study was to determine the effects of Hexabromocyclododecane (HBCD) on the seedling emergence and growth of six species of non-target plants. The experimental design for this study consisted of a negative control and five treatment groups. Each group had four replicate pots with ten seeds planted in each pot. Application of test concentrations of HBCD was made by soil incorporation to each treatment group prior to the planting of seeds. The nominal test substance

concentrations were 0, 40, 105, 276, 725, 1,904, and 5,000 mg of HBCD per kilogram of dry soil (mg HBCD/kg). The mean measured test levels were 0 (Negative Control), 31.3, 97.8, 297, 764, 2,230, and 6,200 mg HBCD/kg dry soil.

Seeds were impartially assigned to prelabeled growth pots on the day of test initiation. The replicate pots were placed in a randomized block design on a greenhouse table after planting. Observations of emergence were made on Days 7, 14, and 21. A general assessment of seedling condition was made on Day 7, while observations of height, shoot dry weight, and assignment of plant condition scores were made only on Day 21.

There were no apparent effects on any endpoint for any of the six species tested. Statistical analyses indicated that there were no significant differences (Dunnett's test, $p>0.05$) between the control and treatment group mean emergence, survival, height, or weight for corn, cucumber, ryegrass, soybean and tomato. On day 21, onion showed a statistically significant difference (Dunnett's test, $p<0.05$) between the control and the 276 mg HBCD/kg treatment group mean survival. This significant difference was not considered dose-responsive, and not attributed to treatment, as no statistical differences were noted at higher concentrations tested (Dunnett's test, $p>0.05$). There were no statistically significant differences (Dunnett's test, $p>0.05$) between the control and signs of treatment-related phytotoxicity observed on seedlings of any species at any test concentration.

No effects from soil incorporation of HBCD were observed on seedling emergence, survival, or growth for any of the six plant species tested. Therefore, the NOEC for emergence and growth of all seedlings in this study was determined to be 5,000 mg of HBCD/kg, which was the highest nominal soil concentration tested.

Porch et al. 2002. Hexabromocyclododecane (HBCD): A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants. Project Number: 439-103. Wildlife International, Ltd. Easton, MD.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
28.12.2004 (21)

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

Type : artificial soil
Species : Eisenia fetida (Worm (Annelida), soil dwelling)
Endpoint : mortality
Exposure period : 28 day(s)
Unit : mg/kg soil dw
NOEC : >= 4190 measured/nominal
LC50 : > 4190 measured/nominal
Method : EPA OPPTS 850.62
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : Earthworm (Eisenia fetida) Survival and Reproduction
(Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA,

OECD and GLP guidelines.

The artificial soil used in this study was characterized as sandy loam with an 80% sand, 8% silt, and 12% clay content. Nominal test concentrations were 0, 78.5, 157, 313, 625, 1,250, 2,500 and 5,000 mg HBCD/kg of dry soil. Mean measured concentrations at day 28 were <1.28 (Control), 61.2, 145, 244, 578, 1,150, 2,180, and 4,190 mg HBCD/kg of dry soil. Mean measured concentrations at day 56 were 56 Days: <1.35 (Control), 51.5, 128, 235, 543, 1,070, 2,020, and 3,990 mg HBCD/kg of dry soil. Measured tissue concentrations at day 28 were <0.200 (control), 3.40, 7.32, 16.8, 15.3, 53.0, 71.2, and 150 mg HBCD per gram of tissue.

After 28 days of exposure to HBCD, percent mortality of the adult worms was 0, 5, 0, 0, 0, 0, 3, and 0% in the 0, 61.2, 145, 244, 578, 1,150, 2,180, and 4,190 mg HBCD/kg groups, respectively. All of the live earthworms were normal in appearance and behavior. No abnormal burrowing or avoidance behaviors were recorded during the first 60 minutes of testing.

The control worms gained an average of 0.418 g per replicate or 10% in replicate mass during the 28 days of adult worm exposure. The mean replicate weight gain of the surviving treatment animals ranged from 0.018 to 0.808 grams. These gains represented average increases of 0.4 to 19% in replicate animal mass over the initial 28 days of exposure.

The average reproduction in the control replicates was 72 juveniles per replicate. The coefficient of variation for the control data was 16%. The average reproduction was 61, 60, 49, 31, 26, 26, and 30 juveniles per replicate for treatment levels 51.5, 128, 235, 543, 1,070, 2,020, and 3,990 mg HBCD/kg, respectively. A statistically significant (p £ 0.05) reduction in reproductive output occurred at treatment levels ³ 235 mg HBCD/kg.

Using the estimated soil Koc and the formula provided in the European Union's Technical Guidance Document, HBCD's calculated BCF in earthworms is:

BCFearthworm = Cearthworm/Csoil
= (Kearthworm-porewater)(RHOsoil)(10+3)/Ksoil-water

(0.15 mg/kg)(1.25 x 10+5)/4,190 mg/kg dry soil = 4.5.

Thus, HBCD did not bioconcentrate in the earthworm.

The 28-Day EC50 (survival) was >4,190 mg/kg. The 28-Day NOEC (survival) was 4,190 mg/kg. The 56-Day EC50 (reproduction) was 771 mg/kg with 95% confidence limits of 225 to 4,900 mg/kg. The 56-Day NOEC (reproduction) was 128 mg/kg.

Aufterheide et al. 2002. Effect of Hexabromocyclododecane on the Survival and Reproduction of the Earthworm, Eisenia fetida. ABC Study No. 47222. ABC Laboratories, Inc., Columbia, Missouri; Wildlife International, Inc., Easton, MD.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
28.12.2004 (11)

Type : artificial soil
Species : Eisenia fetida (Worm (Annelida), soil dwelling)
Endpoint : other: reproduction
Exposure period : 56 day(s)
Unit : mg/kg soil dw
NOEC : = 128 measured/nominal

EC50	:	= 771 calculated
Method	:	EPA OPPTS 850.62
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : Earthworm (Eisenia fetida) Survival and Reproduction (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

The artificial soil used in this study was characterized as sandy loam with an 80% sand, 8% silt, and 12% clay content. Nominal test concentrations were 0, 78.5, 157, 313, 625, 1,250, 2,500 and 5,000 mg HBCD/kg of dry soil. Mean measured concentrations at day 28 were <1.28 (Control), 61.2, 145, 244, 578, 1,150, 2,180, and 4,190 mg HBCD/kg of dry soil. Mean measured concentrations at day 56 were 56 Days: <1.35 (Control), 51.5, 128, 235, 543, 1,070, 2,020, and 3,990 mg HBCD/kg of dry soil. Measured tissue concentrations at day 28 were <0.200 (control), 3.40, 7.32, 16.8, 15.3, 53.0, 71.2, and 150 mg HBCD per gram of tissue.

After 28 days of exposure to HBCD, percent mortality of the adult worms was 0, 5, 0, 0, 0, 0, 3, and 0% in the 0, 61.2, 145, 244, 578, 1,150, 2,180, and 4,190 mg HBCD/kg groups, respectively. All of the live earthworms were normal in appearance and behavior. No abnormal burrowing or avoidance behaviors were recorded during the first 60 minutes of testing.

The control worms gained an average of 0.418 g per replicate or 10% in replicate mass during the 28 days of adult worm exposure. The mean replicate weight gain of the surviving treatment animals ranged from 0.018 to 0.808 grams. These gains represented average increases of 0.4 to 19% in replicate animal mass over the initial 28 days of exposure.

The average reproduction in the control replicates was 72 juveniles per replicate. The coefficient of variation for the control data was 16%. The average reproduction was 61, 60, 49, 31, 26, 26, and 30 juveniles per replicate for treatment levels 51.5, 128, 235, 543, 1,070, 2,020, and 3,990 mg HBCD/kg, respectively. A statistically significant (p £ 0.05) reduction in reproductive output occurred at treatment levels ³ 235 mg HBCD/kg.

Using the estimated soil Koc in Table 1 and the formula provided in the European Union's Technical Guidance Document, HBCD's calculated BCF in earthworms is:

BCFearthworm = Cearthworm/Csoil = (Kearthworm-porewater)(RHOsoil)(10+3)/Ksoil-water

(0.15 mg/kg)(1.25 x 10+5)/4,190 mg/kg dry soil = 4.5.

Thus, HBCD did not bioconcentrate in the earthworm.

The 28-Day EC50 (survival) was >4,190 mg/kg. The 28-Day NOEC (survival) was 4,190 mg/kg. The 56-Day EC50 (reproduction) was 771 mg/kg with 95% confidence limits of 225 to 4,900 mg/kg. The 56-Day NOEC (reproduction) was 128 mg/kg.

Aufterheide et al. 2002. Effect of Hexabromocyclododecane on the Survival and Reproduction of the Earthworm, Eisenia fetida. ABC Study No. 47222. ABC Laboratories, Inc., Columbia, Missouri; Wildlife

International, Inc., Easton, MD.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
29.12.2004 (22)

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo	:	In vivo
Type	:	Toxicokinetics
Species	:	rat
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	other TS

Attached document : From "Toxicological Risks of Selected Flame Retardants", National Academy Press, Washington, DC, 2000, pages 54-55:

"Toxicokinetics

No human data on the toxicokinetics of HBCD were located for any route. No toxicokinetic studies via the dermal or inhalaiton exposure routes were reported in experimental animals. However, in a report by Dean and Leong (1977), rats exposed dermally to a high dose of HBCD in saline experienced diarrhea and slight weight loss. This finding indicates that at least some absorption occurs via the dermal route.

In an unpublished study by Vesicol Chemical Corporation (1980), rats administered a single oral dose of 1.93 mg of radiolabeled HBCD elimenated 86% of the dose within 72 hr. (The total dose administered was 7-9 mg/kg body weight.) Absorption from the gastrointetinal tract reportedly occurred rapidly, with a half-life of 2 hr. However, the amount of the absorbed fraction was not reported. HBCD was reported to be rapidly metabolized and eliminated in the feces and urine following absorption, with 70% of the administed radioactivity eliminatd in the feces and another 16% eliminated in the urine 72 hr after dosing. A two-compartment model was constructed, with non-adipose tissues in one compartment and adipose tissue in the other. Elimination from the adipose compartment was reported to be slower than elimination from the non-adipose compartment, althougth elimination half-times were not provided in the review. In another stuy by Arita et al. (Marcia Hardy, Albemarle Corporation, Pers. Commun., August 3, 1999), HBCD was orally administered to male Wistar rats (number not reported) in olive oil at 500 mg/kg-d for 5 d. HBCD was found to be present only in adipose tissue, and in none of the other organs examined (i.e., spleen, pancrease, liver, kidneys, and heart). HBCD was found to be excreted in the feces, with an average of 32-35% of the cumulative administered dose excreted. No HBCD was found in the urine. Although differences in study design, including the test vehicle and the analytic methods used, may account for some of the difference in the results, both studies by Vesicol Chemical Corporaiton (1980) and Arieta et al. (Marcia Hardy, Albemarle Corporation, Pers. Commun., August 3, 1999) suggeest that following acute oral doses, HBCD is rapidly absorbed from the gastrointestinal tract, distributed primarily to body fat, and eliminated rapidly, primarily in the feces."

For additional details on these studies, please see the following records.

In Vitro/in vivo	:	In vivo
Type	:	Toxicokinetics
Species	:	rat
Number of animals		
Males	:	4
Females	:	0
Doses		
Males	:	500 mg/kg bw/d
Females	:	
Vehicle	:	other: olive oil
Route of administration	:	gavage
Exposure time	:	5 day(s)
Product type guidance	:	
Decision on results on acute tox. tests	:	
Adverse effects on prolonged exposure	:	
Half-lives	:	1st.: 2nd.: 3rd.:
Toxic behaviour	:	
Deg. product	:	
Method	:	other
Year	:	1983
GLP	:	no data
Test substance	:	other TS

Attached document :

The excretion of HBCD in urine and feces as well as its distribution to various organs was investigated. The test article was "Pyroguard SR-103", manufactured by Daiichi Kogyo Seiyaku K.K.

A fine suspension of SR-103 in olive oil was prepared by miing well to homogeneity in a mortar. The dose was 500 mg/kg/d for 5 consecutive days (n= 4 male Wistar rats). The dose volume was 100 mg/ml. Urine and feces were coleected separately by housing the rats in glass cages. Twenty-four hours after the last administration, the rats were sacrifices and the spleen, liver, pancreas, kidneyss, heart and fatty tissue collected. Gas chromatography with an FID detector was used for quantification. The limit of quantification was about 5 ug/ml in urine and about 20 ug/ml in the fecal and organ homogenates.

The average daily rate of excretion in the feces was 29-37% of the dose. The cummulative excretion was roughly constant at 32-35% with respect to the cummulative administered amount. Urinary excretion was not observed.
No evidence for the presence of metabolites was observed in urine or feces.

A separate study with isolated intestinal loop (upper jejunum) indicated about 12% of the dose was detectable in the intestinal tissue and the amount remaining in the lumen loop "small".

The test article was detected only in the adipose tissue after dosing for 5 days. The level in adipose tissue was 0.3-0.7 mg/g fat.

R. Arita, K. Miyazaki and S. Mure. 1983. Metabolic test of hexabromocyclododecane. Department of Pharmacy, Hokkaido University Hospital. Japan.

28.12.2004 (24)

In Vitro/in vivo	:	In vivo
Type	:	Toxicokinetics
Species	:	rat

Number of animals
Males :
Females :
Doses
Males : 7-9 mg/kg
Females : 7-9 mg/kg
Vehicle : other: olive oil
Route of administration : gavage
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st: 27 hour(s)
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : other
Year : 1980
GLP : no data
Test substance : other TS: 14C; probably gamma

Attached document : A single oral dose (7-9 mg/kg) of 14C-HBCD was administered to male (n=2) and female (n=8) rats. Based on the starting material and the melting point of the final product described in the report, the test article appeared to be composed of the gamma isomer. The rats were sacrificed 8, 24, 48 and 72 hours (females) and 48 hours (males) after dosing. One female rat served as control. Urine and feces were collected daily, blood samples from 4 animlas were dollected during the first 24 ours. Tissue samples were collected at the time of sacrifice.

Total 14C-activity was determined via liquid scintillation and the parent molecule and metabolites were distinguished by thin layer chromatography in extracts of feces and urine.

HBCD appeared to be well absorbed from the gastrointestinal tract and extensively metabolized prior to elimination in feces (primary route) and urine. The estimated absorption half-life was 2 hours; peak radioactivity was detected in blood 4 hours post-dosing. The pharmacokinetics of the gamma stereoisomer appeared to follow an open two-compartment model. The central compartment was described as liver, lung, kidney, heart, muscle, gonads, uterus, spleen, and brain; the peripheral compartment was described as adipose tissue. Roughly 80 - >90% of the gamma stereoisomer was eliminated within 3 days following a single oral dose, with an apparent half-life of elimination of 27 hours. Only metabolites were detected in feces and urine - no parent molecule was detected. Thus, the gamma isomer appears to be extensively metabolized prior to excretion. In contrast, only unmetabolized gamma isomer was detected in adipose tissue.

In females at 8, 24, 48 and 72 hours post-dosing, the total 14C-activity detected in tissues of female rats was ~43, 24,18 and 17% of the dose, respectively. In male rats at 48 hours post-dosing (the only time point investigated in males), the 14C-activity was ~10% of the dose. Similarly, that detected in feces from female rats was ~4, 65, 54 and 77% at 8, 24, 48 and 72 h. Urine contained 0.1, 6, 18 and 15% of the dose at the same time intervals. Feces from male rats at 48 hours contained ~94% of the dose while urine from male rats contained ~15%. At 48 hours post-dosing approximately 81% of the dose was detected in feces and urine of female rats. Thus, at 48 hours post-dosing approximately 86% of the dose was recovered in the tissues, feces and urine from female rats whereas 119% was recovered from males. The lower 48 hour recovery from this group of

female rats is largely accounted for by the lower fecal content (43%) of radioactivity collected during the 0-24 hours post-dosing. By 48 hours post-dosing, females sacrificed at 24 hours, had a total fecal 14C-content of 65% of the dose, and females sacrificed at 72 hours had a total fecal 14C-content of 62% of the dose. Substituting the average value, 63%, from these two groups for the 43% value used to calculate overall recovery, the total per cent of the dose accounted for at 48 hours becomes feces (74%), urine (17%) and tissues (18%) or 109% of the dose. It appears likely that some unknown factor resulted in the lower 0-24 hour percent recovery from feces, and that percent of dose present in tissues, feces and urine 48 hours post-dosing is similar in male and female rats.

Caution is urged in interpreting this data due to the small sample size and the brief nature of the final report.

C.C. Yu and Y. H. Atallah. Velsicol Chemical Corporation, Laboratory Report. September 17, 1980.

28.12.2004 (25)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	> 10000 mg/kg bw
Species	:	rat
Strain	:	other: albino
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other: corn oil
Doses	:	0, 10000
Method	:	other: Hagan et al. 1959
Year	:	1978
GLP	:	no
Test substance	:	no data
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

28.12.2004 (26)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	> 200 mg/l
Species	:	rat
Strain	:	Wistar
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other: material was used as received
Doses	:	200 mg/l
Exposure time	:	1 hour(s)
Method	:	other
Year	:	1978
GLP	:	no
Test substance	:	no data
Attached document	:	Inhalation LC50

Albino rats in groups of 10 (5M:5F), 233-292 g, were exposed to a concentration of 200 mg/L, the highest possible chamber concentration, for

1 hour and observed for two weeks. No deaths occured, and no gross changes were observed.

The material was not toxic by inhalation under the conditions of this test.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
28.12.2004 (26)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : > 8000 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 6
Vehicle : other: material used as received
Doses : 500, 2000, 5000, 8000 mg/kg bw
Method : other: Hagan 1959
Year : 1978
GLP : no
Test substance : no data

Attached document : Acute Dermal Toxicity in Rabbits

Following a 14-day range finding, albino rabbits in groups of 6 (3M:3F), 1/2 abraded, 1.88-2.07 kg bw, highest dose mechanically possible, single application dermally under occulded patch, observed for fourteen days. Material used as received. No animals died on test.

The test material was not toxic dermally to rabbits under the conditions of this test.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
28.12.2004 (26)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : .5 g
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : other: material used as received
PDII : 0
Result : not irritating
Classification : not irritating
Method : other: Draize et al. 1944
Year : 1978
GLP : no
Test substance : no data

Attached document : Primary Dermal Irritation in Rabbits

A group of albino New Zealand rabbits were used. Teh test method was

essentially that of Draize et al. 1944.

Briefly, 0.5 g of test material was applied to clipped aras of intact and abraded skin. The abrasions were longitudinal epidermal incisions sufficiently deep to penetrate the stratum corneum, but not so deep as to destroy the integrity of the derma. Applications were made under occulusive patches (2" x 2" gauze, covered by adhesive tape). The entire trunk of each animal was then covered with an impermeable occlusive wrapping, and everted Elizabethan collars were placed on each animal. The wrapping and test material were removed 24 hours after application. The sites were individually examined and scored separately for erythema and dema at 24 and f72 hours. The mean scores for 24 and 72 hour gradings were averaged to determine final irriation indices.

The test material was not irritating. The Primary Irritation Index was 0.0.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
28.12.2004 (26)

Species : rabbit
Concentration : .5 g
Exposure : Occlusive
Exposure time : 4 hour(s)
Number of animals : 6
Vehicle : other: material used as received
PDII : 0
Result : not irritating
Classification : not irritating
Method : other: Draize et al. 1944
Year : 1978
GLP : no
Test substance : no data

Attached document : Dermal Corrosion in Rabbits
49 CFR 173,240(a)(1)

A group of 6 albino New Zealand rabbits, 1,8-2.4 kg, were used. The test method was essentially that of Draize et al. (1944).

Briefly, 0.5 g of the test material was applied to clipped areas of intact skin. Applications were made under occulusive patches (2" x 2" gauze, covered by adhesive tape). The entire trunk of each animal was then coverved with an impermeable occulusive wrapping, and the animal immobilized. The wrapping and test material were removed 4 hours following application. The sites were individually examined and scored for erythema and edema at 4 and 48 hours. The mean scores for each time period were averaged to determine the final irritation indices. Corrosiveness seen at 4 and/or 48 hours alone indicates a corrosive material.

The test material was not corrosive. The Primary Irritation Index was 0.0.

Flag : Critical study for SIDS endpoint
15.12.2004 (26)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : .1 g
Dose : .1 other: g
Exposure time : 24 hour(s)
Comment : rinsed after (see exposure time)

Number of animals	:	6
Vehicle	:	other: material used as received
Result	:	not irritating
Classification	:	not irritating
Method	:	other: Draize et al. 1944
Year	:	1978
GLP	:	no
Test substance	:	no data

Attached document : Primary Eye Irritation in Rabbits (FHSA)

New Zealand rabbits without ocular defects were used. The method was essentially that of Draize et al. 1944.

Briefly, the test substasnce was instilled in the right eye of each animal; the left eye was untreated and served as a control. The treated eyes of all rabbits remained unwashed for 24 hours. Readings were made at 1, 2, and 3 days after treatment. Additional reading were made on days 4-7 as needed.

Mild transient occular irritation was noted at day 1, which decreased progressively over days 2 and 3, and had completed dissapeared by day 4. The scores indicate this material is not irritating according to EU regulations.

Flag : Critical study for SIDS endpoint

15.12.2004 (26)

5.3 SENSITIZATION

Type	:	Mouse local lymphnode assay
Species	:	mouse
Number of animals	:	6
Vehicle	:	other: DMF
Result	:	not sensitizing
Classification	:	not sensitizing
Method	:	other: OPPTS 870.2600
Year	:	2003
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : Contact sensistization potential via the local lymph node assay (including primary irritancy screen) using CBA/J mice. (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of equal parts of the commercial materials produced by Albemarle Corporation, Dead Sea Bromine Compounds, and Great Lakes Chemical Corporation. Its composition was 8.68% alpha, 6.12% beta and 85.10% gamma. This study was performed according to Good Laboratory Practices, OPPTS 870.2600, and OECD 429.

The Local Lymph Node Assay asseses the potential of test materials to cause contact sensitization by measuring hte lymphocyte proliferative responses from auricular lymh nodes following topical application of the test materials to mouse ears. Test materials that elicit a Stimulation Index (SI) >=3 (i.e., 3-fold greater proliferation than control animals) should be considered positive for dermal sensitization potential.

All mice received one of three concetntrations of HBCD (2%, 20% or 50% w/v) or DMF (dimethylformamide) on days 1-3 (n=6 mice/group). HCB (alpha-hexyl cinnamaldehyde), a moderate contact sensitizer, was

evaluated concurrently as a positve dermal sensitizstion control. The test materials were administered to the dorsal surface of both ears (25 ul/ear). On day 6, all mice received ano intravenous tail vein injection of phosphate buffered saline containing 20 uCi of 3H-thymidine. Uptake into the auricular lymph nodes draining the site of chemical application was measured 5 hours later. Body weight data were unremarkable and minor increases in ear thichness were noted suggesting slight irritation following applications of 20% and 50% HBCD. There were no indications that HBCD possesses dermal sensitization potential. SI values were consistently around 1.0 at all doses tested. Lymphocyte proliferation by DMF, vehihcle treated mice (2015 dpm) was higher than historical laboratory values commonly observed using acetone and olive oil as a vehicle. This is not inconsistent with that reported in the literature for this vehicle. HCA adminstrations (30% v/v) elicited prolifeation that waes 3-fold greater than that of vehicle controls thus dtecting the moderate contact sensitization potential in this study. On the basis of these results, HBCD would not be considered to have contact sensitization potential.

Woolhiser M and Anderson P. 2003. Hexabromocyclododecane: Contact sensistization potential via the local lymph node assay (including primary irritancy screen) using CBA/J mice. Study ID 031013. Toxicology & Envionmental Research and Consulting. The Dow Chemical Company. Midland, MI

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
15.12.2004 (27)

Type : Guinea pig maximization test
Species : guinea pig
Concentration : 1st: Induction 5 % intracutaneous
2nd: Induction 250 mg occlusive epicutaneous
3rd: Challenge 250 mg occlusive epicutaneous
Number of animals :
Vehicle : other: corn oil
Result : not sensitizing
Classification : not sensitizing
Method : other: EPA 40CFR798.4100, OECD 406
Year : 1996
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : HBCD Maximization Test in Guinea Pigs
(Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of equal parts of the commercial products manufactured by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. Its composition was 8.5% alpha isomer, 6% beta isomer, and 79.1% gamma isomer.

HBCD was administered in 2 stages (induction and challenge doses) to male guinea pigs (Group 1, n=10; Group 2 n=20). The induction doses were administered to the interscapular region as intradermal injections (phase I) and topical applications (phase II). The challenge dose was administered as topical applications to the flanks of each animal. Phase I of the induction doses consisted of three pairs of intradermal injections of 1) Freund's adjuvant and corn oil (50:50), 2) the test article at a concentration of 5% in corn oil, or 3) the test article at a concentration of 5% in the 50:50 corn oil/Freund's adjuvant solution which were adminsitered to each treated animal (Group 2). The control animals (Group 1) received the same regimen but the test article was omitted. After a period of 7 days, the treated animals (Group 2) received topical applications of neat test article (moistened in corn oil) on the previously

treated interscapular sites.

Following a two week rest period, the challenge doses were administered. Each animal received a topical application of neat tet article (moistened with corn oil) on the left flank and an empty Hilltop Chamber on the right flank. Subsequent examination of the test sites indicated the test article produced no erythema or edema in any animal at 24, 48, 72, 96 or 120 hours after the challenge dose. Based on the percentage of animals responding (0%), HBCD was consided a non-sensitizer.

M. Wenk. Hexabromocyclododecane Maximization Test in Guinea Pigs. 1996. M96AO61.1X64. Microbiological Associates, Inc. Rockville, MD.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
15.12.2004 (28)

Type : Patch-Test
Species : human
Number of animals :
Vehicle :
Result : not sensitizing
Classification : not sensitizing
Method : other
Year : 1972
GLP : no data
Test substance : other TS

Attached document : The test samples were Tyvek T-12 with 10% HBCD. One inch squares of the test samples were applied to the arms of 10 men and to the arms or legs of ten women and held in place with Dermicel tape for six days. After a two-week rest period, new patches were applied for 48 hours as a challenge test for skin sensitization. Skin under the patches was examined at two and six days after the first application and on removal of the challenge patch. No skin reactions were observed on any subject at any examination

McDonnell, M. 1972. Haskell Laboratory Report No. 185-72. Haskell Laboratory for Toxicology and Industrial Medicine.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
20.12.2004 (29)

Number of animals :
Vehicle :
Result :
Classification :
Method :
Year :
GLP : no data
Test substance : other TS

Attached document :

Five sensitization studies have been conducted on HBCD; three in guinea pigs, one in mice, and one in human volunteers. The 1997 guinea pig maximization test performed by BFRIP was negative. Two studies in the literature (Momma et al. Pharmacometrics, 1985, 29:981-986 and Nakamura et al. Contact Dermatitis, 1994, 31:72-85) reported positive results; the test article appears to have been an HBCD product produced in Japan. The patch test in human volunteers was negative. The 2003 mouse local lymph node assay, performed by BFRIP to clarify these results, was negative.

The reason for the discrepancy between these results is not apparent. However, the negative results in the 1997 BFRIP test using the highest possible concentration for topical induction and challenge, raise questions about the potential for HBCD to produce even a mild sensitization reaction in humans. The methodologies used in these 3 sensitization tests are provided in the table below.

Comparison of the methodology used in 3 guinea pig skin sensitization studies conducted on HBCD.

	BFRIP 1995	Monma 1985	Nakamura 1994
INDUCTION - ID			
VOLUME	0.1 ml	0.05 ml	Assume 0.05 ml ?
CONCENTRATION	5%	0.05, 0.5, 5%	0.5, 5%
DOSE	0.005 mg	0.000025, 0.00025, 0.0025 mg	0.00025, 0.0025 mg
VEHICLE	Corn oil	Olive oil	Olive oil
INDUCTION -TOPICAL			
AMOUNT	500 mg	200 mg	Assume 200 mg ?
CONCENTRATION	100%	25%	25%
DOSE	250 mg	50 mg	50 mg
VEHICLE	Corn oil*	Vaseline	Petrolatum
CHALLENGE			
VOLUME/AMOUNT	500 mg	0.02 ml	0.1 ml
CONCENTRATION	100%	0.005,0.05,5%	0.05,0.5,5%
DOSE	250 mg	0.000001, 0.0001, 0.001 mg	0.00001, 0.00005, 0.0005, 0.005 mg
VEHICLE	Corn oil*	Acetone	Acetone

* Only moistened with corn oil.

McDonnell, M. 1972. Haskell Laboratory Report No. 185-72. Haskell Laboratory for Toxicology and Industrial Medicine)
Momma et al. Pharmacometrics, 1985, 29:981-986.
Nakamura et al. Contact Dermatitis, 1994, 31:72-85.
M. Wenk. Hexabromocyclododecane Maximization Test in Guinea Pigs. 1996. M96AO61.1X64. Microbiological Associates, Inc. Rockville, MD.
Woolhiser M and Anderson P. 2003. Hexabromocyclododecane: Contact sensistization potential via the local lymph node assay (including primary irritancy screen) using CBA/J mice. Study ID 031013. Toxicology & Envionmental Research and Consulting. The Dow Chemical Company. Midland, MI

Reliability : (4) not assignable
29.12.2004 (30) (31)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	28 Days
Frequency of treatm.	:	Once daily
Post exposure period	:	14 Days
Doses	:	0, 125, 350, 1000 mg/kg/d
Control group	:	yes, concurrent vehicle
NOAEL	:	= 1000 mg/kg bw
LOAEL	:	> 1000 ml/kg bw
Method	:	OECD Guide-line 407 "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-d Study"
Year	:	1996
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : A 28-day repeated dose oral toxicity study of HBCD in rats. (Sponsor: ACC Brominated Flame Retardant Industry Panel)

This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc.

HBCD, in the vehicle corn oil, was administered orally by gavage to three groups of Sprague-Dawley Crl: CD BR rats for a period of 28 consecutive days. Dose levels were 125 (low), 350 (mid), or 1,000 (high) mg/kg/day, administered at dosage volume of 5 ml/kg. The test groups consisted of 6 males and 6 females in the 125 and 350 mg/kg/day groups and 12 males and 12 females in the 1000 mg/kg/day group. A concurrent control group comprised of 12 males and 12 females received the vehicle, corn oil, for 28 consecutive days at a dosage volume of 5 ml/kg. At the end of the dosing period, 6 animals/sex/group were sacrificed and necropsied. The remaining 6 animals/sex in the control and 1000 mg/kg/day groups remained on-test untreated for a 14-day recovery period. At the end of the recovery period, all animals were sacrificed and necropsied.

Animals were observed twice daily for mortality and moribundity. Clinical signs were recorded daily. Body weight and food consumption were measured weekly. Functional observational battery and motor activity evaluations were performed during weeks -1 (pretest), 3, and 5 (recovery). Samples for hematology and serum chemistry evaluations were collected at the primary (28 day) and recovery (42 day) sacrifices. Complete necropsies were performed on all rats. The brain, liver, kidney, heart, spleen, testes and epididymus or ovaries, adrenal glands, and thymus from all animals were weighed at each sacrifice. Approximately 40 tissues were collected and preserved at each necropsy from all animals. The following tissues were examined microscopically from the control and high dose animals: liver, kidney, heart, spleen, testes (males), prostate (males), seminal vesicles (males), epididymus (males), ovaries (females), adrenal glands, thymus, bone with marrow (sternebra), brain, stomach, cecum, duodenum, ileum, jejunum, lymph node, peripheral nerve (sciatic), spinal cord, lung, trachea, uterus (females), urinary bladder, and all gross lesions. The lungs, liver, kidneys, stomach, thyroid, gross lesions and target organs were examined in all dose levels.

Survival was not affected by administration of the test article. All animals survived to the scheduled sacrifice. Clinical signs observed during the study were nonspecific, low in incidence, non-dose-related and not considered related to test article.

Body weights, weight gain and food consumption of treated animals were compared statistically by sex and treatment day to their respective control groups ($p < 0.05$ or 0.01) and were not affected by treatment. No statistically significant differences in body weight between control and treated animals were detected with the exception of an increase in mean female body weight in the 350 mg/kg/day group during week 2 of treatment. Mean female body weight at that time point was 196 g versus 179 in the control group. No statistically significant differences in body weight gain between control and treated animals were detected with the exception of a decrease in mean male body weight gain in the 1,000 mg/kg/day recovery group during week 1 of recovery. Mean male body weight gain at that time point was 21 g versus 31 in the control group; mean male body weight was not statistically different from the control mean. No statistically significant differences in food consumption between control and treated animals were detected with the exception of an increase in mean female food consumption in the 350 mg/kg/day group during weeks -1, 1, and 2 of treatment. Mean female food consumption at that those time points were 18, 17 and 17 g versus16, 15 and 15 g in the control group, respectively.

Functional observation battery and motor activity results from treated animals were compared statistically by sex and treatment day to their respective control groups ($p < 0.05$). These parameters were not affected by treatment with the test article. No statistically significant differences were observed between treated and control animals at any time point.

No statistically significant differences between treated and control animals were found for hematology parameters with the exception of an increase in the mean activated partial thromboplastin time in the 1000 mg/kg/day males on week 4 and a decrease in the mean prothrombin time in the 1000 mg/kg/day females on week 4. These statistical differences were not of toxicological significance.

No toxicologically significant effects on serum chemistry values related to test article administration were observed at the 28-day primary and 42-day recovery sacrifice. Scattered instances of statistically significant differences between treated and control animals were detected for some serum chemistry parameters at the 28-day primary sacrifice. These scattered statistical differences were not considered toxicologically significant because the statistical differences occurred: in the absence of a dose response, in the absence of the accompanying clinical chemistry changes expected, in the opposite direction from what occurs in a toxic state, in a direction which is without physiologic significance, or due to potential interference with the laboratory method. No statistically significant differences in serum chemistry parameters were detected between groups at the 42-day recovery sacrifice.
No gross lesions that could be attributed to the test article were detected at either necropsy. Gross lesions were nonspecific, low in incidence, non-dose-related and considered incidental.

No microscopic lesions that could be attributed to the test article were detected on histopathologic exam. Microscopic changes were nonspecific, low in incidence, non-dose-related and considered incidental.

No statistical significant differences in organ weight or organ to body weight ratios were detected between control and treated animals with one exception. Absolute liver weights were statistically significantly increased with respect to control means at the 28-day sacrifice in males in the high dose and females in the mid and high dose. Liver to body weight ratios in mid and high dose males and low, mid and high dose females were statistically significantly increased at the 28-day sacrifice. At the recovery

sacrifice, male absolute and liver to body weight ratio were statistically comparable to the control mean whereas female absolute liver weights and liver to body weight ratio were statistically significantly increased with respect to control mean. The difference in absolute liver weight between control and treated females was less pronounced at the end of the recovery period, indicating the increase in liver weight was reversible in females as well as males. In the absence of test article related histopathologic and serum chemistry changes, increases in liver weight are considered an adaptive, rather than a toxic response, are not uncommon in the rat, and are most likely the result of microsomal induction.

In conclusion, no systemic toxicity was observed at any dose level. Based on the results of this study, the NOAEL (No Observed Adverse Effect Level) of HBCD administered orally to male and female rats for 28 consecutive days was 1,000 mg/kg/day.

Chengelis C. 1996. A 28-day repeated dose oral toxicity study of HBCD in rats. Study No. WIL-186004. WIL Research Laboratories, Inc. Ashland, OH.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
29.12.2004 (32)

Type : Sub-chronic
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : 90 Days
Frequency of treatm. : Once Daily
Post exposure period : 28 Days
Doses : 0, 100, 300, 1000 mg/kg/d
Control group : yes, concurrent vehicle
NOAEL : = 1000 ml/kg bw
Method : EPA OPPTS 870.3100
Year : 2001
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document : An Oral (Gavage) 90 Day Toxicity Study of HBCD in Rats. (Sponsor: ACC Brominated Flame Retardant Industry Panel)

This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc.

The test article, a composite of three lots of commercial hexabromocyclododecane (HBCD), was administered by oral gavage in corn oil once daily to four groups of Crl:CD(SD)IGS BR rats (n=15/sex/group) at dose levels of 0 (control), 100 (low), 300 (mid) and 1000 (high) mg/kg/day seven days per week for 90 days. The dosage volume was 5 ml/kg. The control animals received the vehicle, corn oil, only. At the end of the 90-day treatment period, 10 animals/sex/group were euthanized and necropsied. The remaining rats continued on test untreated for a 28-day recovery period prior to necropsy.

In addition to the main toxicology groups, two satellite groups of 20 animals/sex/group were treated concurrently in an identical manner at dose levels of 0 or 1000 mg HBCD/kg/day for up to 90 days. Body weights were recorded weekly. Two animals/sex/group were euthanized on study days 2, 6, 9, 13, 20, 27, 55, 89, 104 and 118, and blood and body fat

(mesenteric and/or omental) were collected. The body fat was analyzed for HBCD content.

Animals in the main toxicology groups were observed twice daily throughout the study for mortality and morbidity. Body weights and food consumption were measured weekly. Blood was collected at study weeks 3 (n=5/sex/group), 13 (n=10/sex/group) and 17 (n=5/sex/group) for hematology, serum chemistry and hormone (T3, T4 and TSH) measurements. Urine was collected prior to each necropsy, at study weeks 13 and 17, for urinalysis. Ocular examinations were performed prior to study initiation and during study weeks 12 and 15. Functional Observational Battery and Locomotor Activity evaluations were performed on 5 animals/sex/group prior to study initiation, during the last week of test article administration (study week 13), and during the recovery period. An examination of vaginal cytology (for estrus cycle determinations) was performed on study days 69-90. At each necropsy, sperm motility/viability, morphology, and number were assessed. Complete necropsies were performed on all animals. Approximately 40 organs or tissues/animal were collected and preserved. The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, thyroids with parathyroids, and uterus with cervix were weighed. Paraffin sections of tissues stained with hematoxylin and eosin from the control and 1000 mg/kg/day dose groups and the liver, lungs and thyroid glands in the 100 and 300 mg/kg/day doses, and gross lesions from all animals were examined under the light microscope. Livers from five randomly chosen animals/sex from the control and 1000 mg/kg/day dose groups were examined microscopically using Oil Red O or periodic acid Schiff's (PAS) reagent for evidence of lipid accumulation or glycogen accumulation/depletion, respectively. Statistical comparisons by sex and treatment day were made between the control and treated animals where indicated ($p<0.05$).

No test article-related effect on mortality occurred. Clinical signs were non-specific, low in incidence, non-dose-related and not related to test article administration. No test article-related changes occurred in body weight, food consumption, Functional Observational Battery or Locomotor Activity. No test article-related effects on hematologic parameters were noted. No test article-related ocular lesions were detected at the ophthalmic exams. No test article-related changes were noted on the estrus cycle as determined by vaginal cytology, or on sperm motility/viability, morphology, and number. Instances of statistically significant differences between control and some treatment groups were detected at study week 13 in the clinical chemistry data, hormone data, organ weight data and histology findings. They were considered secondary to hepatic enzyme induction or were otherwise not considered adverse effects of treatment as discussed further below.

Statistically significant ($p<0.05$) test article-related serum clinical chemistry changes at week 13 include an increase in albumin (all dose levels for males), total protein (all dose levels for females and 1000 mg/kg/day for males), globulin (300 and 1000 mg/kg/day for females), and chloride (all doses for both sexes). In addition, increased gamma glutamyltransferase levels were noted in the 1000 mg/kg/day group ($p<0.05$). Thyroxine (T4) levels were decreased at study week 13 compared to the control mean in all male dose groups and the 300 and 1000 mg/kg/day dose females ($p<0.05$). There were no corresponding statistical effects on T3 and TSH.

While potentially test article-related, the changes in serum chemistry parameters were not of sufficient magnitude to be adverse, occurred in otherwise clinically normal animals, tended to be within or close to historical control values, and were not present at the end of the recovery period. The increased serum albumin and gamma glutamyltransferase

levels were considered related to the increased in liver weight in treated animals that was believed due to enzyme induction. Hepatic GGT is inducible in the rat (Teschke et al 1983. Gut. 24(7):625-30; Chandar and Nagarajan 1984. Biochem Int. Jan;8(1):41-8), and this induction has been shown to increase serum GGT levels in the rat (Goldberg 1980. Crit Rev Clin Lab Sci. 12(1):1-58; Teschke et al. 1983. Gut 24(7):625-30; Nishmura and Teschke 1982. Biochem Pharmacol. Feb 1;31(3):377-81; Satoh et al. 1982. J Pharmacol Exp Ther. Jun;221(3):795-800). The increase in serum chloride was considered secondary to be presence of free bromide in the test article preparation that interfered with the chloride determination methodology. The decrease in T4, which was also reversible, was considered related to the increased liver weights in that induction of hepatic UDPG is known to enhance the elimination of T4. The rat is particularly sensitive to hepatic enzyme induction.

The incidence of observations noted at gross necropsy was low and was not accompanied by evidence of frank organ damage. On histopathologic examination, mild findings were detected in both the control and treated groups. Potential test article?related histologic changes were identified in the liver and thyroid glands, but these were not considered to be indicative of frank toxicity. The liver changes in male rats at the 90-day necropsy (Study Week 13) were characterized as minimal hepatocellular vacuolation and occurred in 10% of control males and ~50% of the males at 100, 300 and 1000 mg/kg/day. Thus, there was no increase in incidence with dose. Minimal hepatocellular vacuolation was also detected in females in the control and treated groups without a clear dose response (3 to 4/10 animals per group). Mild and moderate vacuolation was detected in females only in the 300 (1/10) and 1000 mg/kg/day (2/10) dose groups. Minimal to mild hepatocellular hypertrophy was also detected only in the 1000 mg/kg/day group (5/10) females. Minimal thyroid follicular cell hypertrophy was detected 1/10, 1/10, 5/10 and 7/10 males in the control, 100, 300 and 1000 mg/kg/day groups, respectively and in 4/10 and 3/10 females in the 300 and 1000 mg/kg/day groups respectively. In addition, mild thyroid follicular hypertrophy was detected in 4/10 females in the 1000 mg/kg/day group.

The histologic changes in the liver were accompanied by an increase in liver weight. In contrast there were no statistically significant changes in thyroid weight (absolute, relative to body weight and relative to brain weight). At study week 13, mean liver weights in all dose levels of both sexes (absolute, relative to body weight and relative to brain weight) were increased compared to the male and female control means ($p<0.05$).

The changes seen in this study, an increase in liver weight, the lack of adverse histologic effects in the liver and the apparent normal functioning of the liver, are consistent with enzyme induction (Amacher et al. 1998. Food Chem. Toxicol. 36:831-830), and hepatic enzyme induction is an adaptive, and not an adverse, effect (Williams and Iatropoulos 2002. Toxicol Pathol. Jan-Feb;30(1):41-53). Hepatocellular vacuolation and hypertrophy, seen in this study, often accompany the increased liver weight caused by liver enzyme induction, and were reversible. At week 17, the liver changes (weight and histology) had at least partially, if not fully, resolved in all treated groups without delayed or long-term toxic effects.

Limited pharmacokinetic studies indicate HBCD is extensively metabolized prior to excretion in the feces and urine. The results of the fat analysis in this study indicate that mammalian system handles the 3 HBCD stereoisomers differently, and may be less efficient at eliminating one stereoisomer over another. (The relative isomer concentrations in adipose tissue at all time points were alpha>>gamma>beta in contrast to the test article composition of gamma>>alpha>beta.) Thus, it should not be unexpected that hepatic enzyme induction occurs with exposure to

substantial doses over a significant period of time, as was the case in the 90-day study.

The histologic changes in the thyroid consisted of a slight increase in the incidence of minimal follicular cell hypertrophy in the high dose males and minimal or mild hypertrophy in the high dose females. It was not readily apparent that these minimal changes were an effect of treatment, and in any event appeared reversible. The follicular cell hypertrophy may have been related to serum T4 levels. Follicular cell hypertrophy is the normal physiological response to reduced to serum T4 levels and is the typical adaptive response of a healthy normally functioning organism acting to maintain serum T4 levels in the normal range.

The mean prostate weight was increased in the 1000 mg/kg/day group males at the primary necropsy. This was not considered to be of toxicological significance since the increase did not persist to the recovery period, there were no correlating histologic findings and no change in seminal quality.

HBCD was detected in the adipose tissue of male and female rats treated with 1000 mg/kg/day for up to 90 days. Isomer-specific analysis showed that the relative isomer concentrations in adipose tissue at all time points were alpha>>gamma>beta which is in contrast to the test article composition (gamma>>alpha>beta). Steady state levels were achieved by study day 27. Levels in male and female rats were similar at all time points and declined during the recovery period.

All the test article-related changes at 100 and 300 mg/kg/day were mild, reversible, generally secondary to hepatic enzyme induction (which is an adaptive not a toxic change) and without effect on the clinical condition of the animals. The findings observed at 1000 mg/kg/day (increased serum gamma glutamyltransferase, increased liver and prostate weights), were also reversible, not associated with specific target organ damage or diminished function and were, therefore, of limited, if any, toxicologic significance. On this basis, the no-observed-adverse-effect level (NOAEL) of HBCD administered to Crl:CD®(SD)IGS BR rats by gavage in corn oil for 90 days is 1000 mg/kg/day.

Chengelis C. An Oral (Gavage) 90 Day Toxicity Study of HBCD in Rats. Study No. WIL-186012. WIL Research Laboratories, Inc., Ashland, Ohio. 2001.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
29.12.2004 (33)

Type : Sub-chronic
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 28 Days
Frequency of treatm. : Daily
Post exposure period : None
Doses : 0, 1, 2.5, 5% Diet
Control group : yes, concurrent no treatment
NOAEL : = 1 %
Method : other
Year : 1969
GLP : no data
Test substance : other TS: Hexabromid S (HBCD formerly manufactured by BASF)

Attached document : 28-Day Feeding Study of HBCD in Rats. (Sponsor: BASF)

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats (10/sex/group) at doses of 0, 1, 2.5 and 5% of the diet for 28 days. Doses calculated from the actual body weights and food consumption in this study are 0, 940, 2410, and 4820 mg/kg body weight/day.

No clinical signs related to treatment were observed at the 1% dose level. Body weights at the 1 and 2.5% dose levels were comparable to the controls. Liver weights (absolute and relative to body weight) were increased at all dose levels, but no microscopic pathology was detected. Thyroid hyperplasia was observed in some animals at all doses, and "very slight numerical development of the follicles and ripening follicles in the ovaries of females" at the high dose (4820 mg/kg/d) was reported. No changes in any other organ related to treatment and no changes in clinical chemistry tests were detected.

The report concluded that "The increased liver weight must be attributed to hyperactivity; hypermetabolism as a result of increased thyroid activity appears probable in view of the observations of the thyroid". Therefore, the increased liver weights were not pathologic: there were no microscopic lesions detected on histopathology and no change in clinical chemistry values.

Zeller H and Kirsch P (1969) Hexabromocyclododecane: 28-day feeding trials with rats. BASF Unpublished Laboratory Report.

Reliability : (2) valid with restrictions
29.12.2004 (34)

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	90 Days
Frequency of treatm.	:	Daily
Post exposure period	:	None
Doses	:	0, 0.16, 0.32, 0.64 and 1.28% Diet
Control group	:	yes, concurrent no treatment
NOAEL	:	= 1.28 %
Method	:	other
Year	:	1970
GLP	:	no data
Test substance	:	other TS: Hexabromid S (HBCD formerly manufactured by BASF)

Attached document : 90-Day Feeding Study of HBCD in Rats (Sponsor: BASF)

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats at doses of 0, 0.16, 0.32, 0.64 and 1.28% of the diet for 90 days. Doses calculated on the actual body weights and food consumption in this study reveals: 0, 120, 240, 470 and 950 mg/kg body weight/day.

Doses up to 0.64% (470 mg/kg/d) produced no adverse clinical signs, no change in body weight, and no change in clinical chemistry results. An increase in the relative liver to body weight ratio was found, and was accompanied by fatty accumulation but no other histologically discernible changes were detected in the liver. Further, no histological changes were found in any other organ. The original report stated that in the "absence of detectable clinico-chemical disturbances or histological changes of the vital organs, it was concluded that the increased liver weight and the fat deposits, both of which were largely reversible when administration of Hexabromid S was stopped, were the result of a temporary increase in the

activity of the liver." Thus, no adverse effect was produced at the highest dose tested, 1.28% of the diet.

Zeller H and Kirsch P (1970) Hexabromocyclododecane: 90-day feeding trials with rats. BASF Unpublished Laboratory Report.

Reliability : (2) valid with restrictions
29.12.2004 (35)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Salmonella microsomal
Test concentration	:	Various
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	other TS

Attached document : HBCD has been tested for mutagenicity in the Ames Salmonella microsomal assay, both with and without metabolic activation, in multiple tests. All results were negative:

Ogaswara S and Hanafusa T. (1993) Report on mutagenicity test on Pyroguard SR-103 using microorganisms;

Baskin A and Phillips, B. (1977) Mutagenicity of two lots of FM-100, Lot 53 and residue of Lot 3322 in the absence and presence of metabolic activation. Industrial Biotest Laboratories, Sponsored by Velsicol Chemical Corporation;

Anonymous. (1979) Mutagenicity test of GLS-S6-41A. Gulf South Research Institute, Sponsored by Ethyl Corporation;

US Environmental Protection Agency (1990) Ames metabolic activation test to assess the potential mutagenic effect of Compound No. 49. Letter from BASF. EPA/OTS Doc #86-900000385;

Simmons V., Poole, D., Newell, G., and Skinner, W. (1976) In vitro microbiological mutagenicity studies for four CIBA-GEIGY Corporation compounds. SRI Project LSC-5702.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
28.12.2004

Type	:	Chromosomal aberration test
System of testing	:	human peripheral blood lymphocytes
Test concentration	:	10, 19, 38, 75, 150, 300 and 600 ug/ml
Cycotoxic concentr.	:	750 ug/ml
Metabolic activation	:	with and without
Result	:	negative
Method	:	EPA OPPTS 870.5375
Year	:	1996
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : In vitro Chromosomal Aberration Test. (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

HBCD was tested in the in vitro mammalian cytogenetic test using human peripheral blood lymphocytes both in the absence and presence of metabolic activation. The assay was performed in two phases. The first phase, the initial chromosome aberration assay, was conducted to establish the dose range for testing and to evaluate the clastogenic potential of the test article. The second phase, the independent repeat chromosome aberration assay, was performed to confirm the test system response to the test article seen in the initial assay.

Dimethylsulfoxide was used as a solvent. In the initial assay, the maximum dose tested was 2,500 ug/ml. Dose levels greater than 2,500 ug/ml were insoluble in treatment medium. Visible precipitate was observed in treatment medium at 750 and 2,500 ug/ml and was soluble but cloudy at dose levels of 75 and 250 ug/ml. The test article was soluble in treatment medium at all other doses tested. In the non-activated portion of the initial assay cells were exposed to the test article continuously for 20 hours; in the S9-activated portion of the initial chromosome aberration assay, cells were exposed to the test article for 4 hrs. Metaphase cells were collected at 20 hrs after initiation of treatment. Dose levels of 2,500 ug/ml in the non-activate study and 750 and 2,500 ug/ml in the S9-activated study were not analyzed for chromosome aberrations due to complete mitotic inhibition. Toxicity (mitotic inhibition) of ~56% was observed at the highest dose level (750 ug/ml) evaluated for chromosome aberrations, in the non-activated study. In the S9-activated study, 13% toxicity was observed at the highest dose level (250 ug/ml) evaluated for chromosome aberrations. No statistically significant increases in chromosome aberrations were observed in either the non-activated or S9-activated test systems relative to the solvent control group regardless of dose level.

Based on the results of the initial assay, an independent repeat chromosome aberration assay was conducted in the absence and presence of an Arochlor-induced S9 metabolic activation system at dose levels of 10, 19, 38, 75, 150, 300 and 600 ug/ml. The test article was soluble but cloudy at 75 ug/ml and was workable in treatment medium at dose levels 150 ug/ml and higher. The test article was soluble in treatment medium at all other concentrations tested. In the independent repeat assay, cells were exposed to the test article continuously for 20 or 44 hr in the non-activated test system and for 4 hours in the S9-activated test system. Metaphase cells were collected for microscopic evaluation in both the non-activated and S9-activated studies at 20 and 44 hrs after initiation of treatment. Toxicity, measured by mitotic inhibition, was ~55% and 94% at the 20 and 44 hr harvests, respectively, at the highest dose levels (600 and 300 ug/ml) evaluated for chromosome aberrations in the nonactivated studies. In the S9-activated studies, toxicity was approximately 71% and 69% at the 20 and 44 hr harvests, respectively, at the highest dose levels (600 and 300 ug/ml) evaluated for chromosome aberrations. The 600 ug/ml dose level in the non-activated 44 hr harvest and in the S9-activated 20 hr harvest was not analyzed for chromosome aberrations due to an insufficient number of scorable metaphase cells. No statistically significant increases in structural chromosome aberrations were observed in either the non-activated or S9-activated studies, regardless of dose level or harvest time. No statistically significant increases in numerical chromosome aberrations were observed in either the non-activated or S9-activated studies at the 44 hr harvest time, regardless of dose level. HBCD was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes.

Gudi, R. and Schadly, E. 1996. Laboratory Study Number G96AO61.342. Microbiological Associates, Inc., Rockville, MD.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
29.12.2004 (36)

Type : other
System of testing : Hamster cells
Test concentration : see freetext
Cycotoxic concentr. :
Metabolic activation :
Result :
Method : other
Year : 1999
GLP : no
Test substance : no data

Attached document : In Vitro Iatrogenic Recombination

The Sp5 and SPD8 cell lines were developed by the paper's authors. The clones used in this study exhibit a spontaneous partial duplication of the hprt gene, resulting in a non-functional hgprt protein. These mutants revert spontaneously to a functional hprt gene phenotype by recombination with a frequency of 1 x 105 reversions/cell generation. This reversion frequency is said to increase by exposure to chemical or physical agents. Treatment with the test substance was for 24 hr at 37 degrees C.

HBCD was tested in vitro in hamster cells (Sp5/V79 and SPD8) in a recombination assay at five doses between 2 and 20 ug/ml plus a control. In the SPD8 cells, HBCD concentrations of 0, 3, 6, 10, 15, and 20 ug/ml resulted in a reversion frequency of 1.0, 0.7, 0.8, 0.9, 1.4, and 1.9, respectively. Cytoxicity was observed at the 20 ug/ml dose. In the Sp5 cells, HBCD concentrations of 0, 2, 5, 10, 15, 20 ug/ml resulted in a reversion frequency of 1.0, 1.0, 0.8, 1.1, 1.4 and 2.2, respectively. Cytotoxicity was not observed. The reversion frequency at the 20 ug/ml dose for the Sp5 and SPD8 cells was statistically different from the control (Student's t test, $p<0.05$). Treatment with HBCD resulted in an ~ maximal 2-fold increase in revertant frequency. (Helleday et al. Brominated flame retardants induce intragenic recombination in mammalian cells. Mutation Research 439 (1999) 137-147). This is a non-standard genetic toxicity test, and its reliability and predictive ability is unknown. This is not a test used by regulatory agencies to assess genotoxicity potential.

Reliability : (4) not assignable
20.12.2004 (37)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : mouse
Sex : male
Strain :
Route of admin. : i.p.
Exposure period : Once
Doses : 0, 500, 1,000 or 2,000 mg/kg bw
Result : negative
Method : OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year : 2000
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Attached document	:	In Vivo Mouse Micronucleus Test (Sponosor: BASF Corporation) The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current OECD guidelines and Good Laboratory Practices. HBCD dose levels administered intraperitoneally to male mice were 0, 500, 1,000 or 2,000 mg/kg body weight. The negative control animals were administered the vehicle, DMSO. Cyclophosphamide and vincristine were used as positive controls and responded as expected. HBCD-treatment did not increase in number of polychromatic erythrocytes containing either small or large micronuclei. Micronuclei formation in HBCD-treated mice was within the same range as that of the concurrent negative control and within the range of historical control data. No evidence of chromosome damaging (clastogenic) effects was observed. There was no indication of any impairment of chromosome distribution in the course of mitosis. HBCD was clearly negative for clastogenicity and the ability to induce spindle poison effects in this mouse micronucleus test. Engelhardt, G and Hoffmann, H. (2000) Laboratory Project Identification: 26M0100/004018. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany.
Reliability	:	(1) valid without restriction
Flag	:	Risk Assessment, Critical study for SIDS endpoint

29.12.2004 (38)

5.7 CARCINOGENICITY

Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	oral feed
Exposure period	:	18 months
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0, 100, 1000 or 10,000 ppm
Result	:	negative
Control group	:	yes, concurrent no treatment
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	other TS

Attached document	:	Male and female mice were fed diets containing HBCD at 0, 100, 1000 or 10,000 ppm for 18 months. There was no evidence of carcinogenicity at any dose level. This study was performed by the Department of Toxicology, National Public Health Research Institute, Biological Safety Test and Research Center, Japan (date not specified). Only a summary of the study is available. The test substance was the commercial HBCD product from Daiichi Kogyo Seiyake K.K. There were 50 males and 50 females per dose level. There was no effect of test article on mortality, clinical signs, body weights or food consumption. A variety of gross lesions/nodules were detected at necropsy which were not correlated with administration of test article. The

main histopathologic change noted was hepatocyte swlling, degenration, ncerosis, vacuole formation and fatty infiltration, and were suspected to be related to hepatic enzyme induction. There was no correlation of these changes with dose level. Various tumors were observed in many organs, but such incidence was sporadic and not due to test article administration.

Reliability : (2) valid with restrictions
28.12.2004 (39)

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	Day 6-19 of Gestation
Frequency of treatm.	:	Once daily, 7 days/wk
Duration of test	:	
Doses	:	0, 250, 500, 1000 mg/kg/d
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	>= 1000 mg/kg bw
NOAEL teratogen.	:	>= 1000 mg/kg bw
Result	:	Not a developmental toxicant
Method	:	EPA OPPTS 870.3700
Year	:	1999
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Attached document : A Prenatal Developmental Toxicity Study of Hexabromocyclododecane (HBCD) in Rats. (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The study was performed according to EPA, OECD and GLP guidelines. This study was required by KEMI (without consultation of the EU Technical Meeting) because KEMI decided the existing study in the literature (Murai et al.) was insufficient.

HBCD was administered in corn oil by gavage to 25 presumed pregnant Crl:CD(SD)IGS Br rats/group once daily from gestation days 6-19 at doses of 0, 250, 500 or 1,000 mg/kg/day. Control animals received corn oil only. Female rats were mated in-house and were treated daily on gestation days 6-19 with HBCD via gavage at dose levels of 0 (vehicle control), 250, 500 or 1000 mg/kg/day at a constant volume of 5 ml/kg. Individual doses were based on the most recent body weight. The day on which evidence of mating was observed was considered day 0 of gestation. Dams were observed daily and maternal body weight and food consumption measured at appropriate intervals. Females were euthanized on day 20 of gestation and necropsied. Gravid uterine and liver weights were recorded. Litters were delivered by cesarean section. The total number of corpora lutea, total number of implantations, early and late resorptions, number and location of all fetuses, and the sex and individual weights of fetuses were recorded. All fetuses were examined grossly. Approximately one-half of the fetuses in each litter were stained with Alizarin Red S and Alcian Blue and evaluated for skeletal/cartilaginous malformations and ossification variations. The maternal day 20 gestation examinations and cesarean

sections, and subsequent fetal evaluations were performed blind to treatment.

No mortality occurred during the course of the study. No treatment-related clinical signs were observed. Body weight gain and food consumption were not adversely affected. No treatment-related findings were detected at necropsy. Intrauterine growth and survival were unaffected by treatment. No treatment-related fetal malformations or developmental variations were observed. The no-effect level (NOEL) for maternal toxicity and developmental toxicity was 1,000 mg/kg/day, the highest dose tested

Stump, D. 1999. A Prenatal Developmental Toxicity Study of Hexabromocyclododecane (HBCD) in Rats. Laboratory Study No.: WIL-186009. WIL Research Laboratories, Inc., Ashland, OH.

Reliability : (1) valid without restriction
Flag : Risk Assessment, Critical study for SIDS endpoint
29.12.2004 (40)

Species : rat
Sex : female
Strain : other
Route of admin. : oral feed
Exposure period : Day 0-20 of Gestation
Frequency of treatm. : Daily
Duration of test : Day 0-20 of Gestation or Day 0 Gestation through 7 wks postpartum
Doses : 0, 0.01, 0.1, or 1% of diet
Control group : yes, concurrent no treatment
NOAEL maternal tox. : >= 1 %
NOAEL teratogen. : >= 1 %
Result : Not a developmental toxicant
Method : other
Year : 1985
GLP : no data
Test substance : other TS

Attached document : Murai et al. 1985 (Pharmacometrics (Japan) 29(6):981-986) identified no reproductive or developmental effects in the rat at doses up to 1% in the diet administered from days 0-20 of gestation. This dose is approximately equivalent to 500 mg/kg/d.

The Murai et al study consisted of a 7 day dose range finding study (n=5 rats/dose group) and a combined teratogenicity-developmental study (n=20/dose group). Doses in the 7 day range finding study were 0, 0.3, 1, 3 or 10 g/kg/day. Doses as high as 10 g/kg/day produced no evidence of toxicity. A statistically significant (P<0.01) increase in liver weight was noted in groups receiving > 1 g/kg/day. Doses for the combined teratogenicity-developmental study were based on this increase in liver weight. In the combined teratogenicity-developmental study, pregnant female rats were fed diets containing 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation. Daily doses were estimated by the authors to be 0, 5, 50 or 500 mg/kg/day and the average total dose/rat/group was estimated to be 0, 0.13, 1.28 or 12.0 g/kg. Rats were observed daily and body weight and food consumption measured. Fourteen rats from each group were sacrificed on day 20 of gestation and their fetuses were examined for toxicity or teratogenicity. Approximately 150 fetuses/dose level were examined for evidence of teratogenicity. All fetuses from all litters were examined for signs of external anomalies. Approximately 2/3 of the fetuses/dam were examined for skeletal abnormalities; the remaining fetuses from each dam were examined for any abnormalities of the internal organs. In addition, six rats from each group were allowed to deliver their litters and growth of the litters was observed until the 7th week post-parturition.

The authors' estimated the doses in the feed were equivalent to 0, 5, 50 or 500 mg HBCD /kg body weight /day. No adverse effects were detected in any treatment group with respect to maternal weight gain, food consumption, or gross appearance of internal organs. The mean liver (absolute and relative to body weight) weight in the 1% group was statistically different (higher) from the control mean. Normal development was seen in neonates carried through to six weeks of age.

There was no adverse effect of treatment on the number of corpora lutea, implants, resporptions, live fetuses, sex ratio, or body or placental weight. No fetal deaths occurred in any group. No external, skeletal or visceral malformations were detected. A few skeletal variations were detected but where of similar types and numbers in the control and treated groups.

There was no significant differences between the control and treated groups in the number of implantation, live newborns, dead newborns, live newborn parturition index. The weaning and survival index was comparable in the control and treated groups. Body weight changes in the newborns was comparable in all groups.

No reproductive or developmental effects where detected in rats at HBCD doses up to 1% in the diet (~500 mg/kg/d) administered from days 0-20 of gestation. Further, normal development was seen in neonates carried through to six weeks of age.

Dose levels: 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation [Murai estimate: 0, 5, 50 or 500 mg/kg/day]. No teratogenic effects. Normal development in neonates carried through age 6 wks. NOEL = 1% of diet.

Murai, T. Kawasaki, H., Kanoh, S. 1985. Studies on the toxicity of insecticides and food additives in pregnant rats - fetal toxicity of Hexabromocyclododecane. Pharmacometrics (Japan) 29(6):981-986).

Reliability : (1) valid without restriction
28.12.2004 (41)

14.12.2004

14.12.2004

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

Type	:	other
In vitro/in vivo	:	In vivo
Species	:	rat
Sex	:	male/female
Strain	:	
Route of admin.	:	
Exposure period	:	
Frequency of treatm.	:	
Duration of test	:	
Doses	:	
Control group	:	
Result	:	See Freetext

Attached document : Two teratology studies on HBCD are available; one published in the literature (Murai et al. 1988; high dose = 1% of the diet) and one recently completed by industry (1999) under current guidelines and Good

Laboratory Practices using the HBCD in commercial production and use (high dose = 1,000 mg/kg/d). Both studies are negative for developmental toxicity. Repeated dose studies (two 28 day studies, one 90 day study, and one 18 month study in a second species) indicate HBCD does not affect the reproductive organs at doses up to 1,000 mg/kg/day. According to the OECD SIDS Manual, when teratology and 90 day studies show no effects on the reproductive system then the requirement for the reproductive endpoint are met. Teratology, 28 day, 90 day and 18 month studies all demonstrate HBCD has no effect on the reproductive system at the limit dose of 1,000 mg/kg/d.

In the 2001 90-Day study, considered the most informative of the subchronic studies mentioned in the preceding paragraph, the following organs of the reproductive tract were weighed at the 90-day and recovery sacrifices: epididymides (total and cauda), ovaries (with oviducts), prostate, testes, and uterus and cervix. These organs plus the semininal vesicles, vagina and vas deferens were examined histologically in animals in the control and high dose groups. Vaginal smears for determination of the stage of estrus were obtained form all females once daily beginning study day 69 through the 90-day necropsy. An assessment of spermatogenesis was performed at the 90-day sacrifice by evaluating sperm motility/viability, morphology and epidiymal and testicular sperm numbers and production rate.

No adverse effect on the estrous cycle was detected in females receiving doses as high as 1,000 mg/kg/d for 90 consecutive days. No adverse effect on spermeogenesis was detected in males receiving doses as high as 1,000 mg/kg for 90 days. No test-article related changes in weight or microscopic effects were noted in the organs of the reproductive tract with the sole exception of an increase in weight of the prostate at 1,000 mg/kg/d on day 90. Mean prostate weight was increased in the 1,000 mg/kg/d dose group compared to the control mean after 90 days of treatment (0.95, 0.99, 1.12, 1.25* g in the control, 100, 300, and 1,000 mg/kg/d, respectively). The mean prostate weight relative to mean body and brain weights was also statistically increased at the high dose on day 90. At the recovery sacrifice, the prostate weights in the control and high dose groups were not statistically different. No test article-related changes in the prostate were detected on microscopic exam at either sacrifice.

28.12.2004

5.9 SPECIFIC INVESTIGATIONS

Endpoint	:	Neurotoxicity
Study descr. in chapter	:	5.4 Repeated Dose Toxicity
Reference	:	Chengelis C. An Oral (Gavage) 90 Day Toxicity Study of HBCD in Rats. Study No. WIL-186012. WIL Research Laboratories, Inc., Ashland, Ohio. 2001
Type	:	other: Functional Observational Battery, Motor Activity
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Oral, gavage
No. of animals	:	10
Vehicle	:	other: corn oil
Exposure period	:	90 day(s)
Frequency of treatm.	:	once daily
Doses	:	0, 100, 300, 1000 mg/kg bw
Control group	:	yes, concurrent vehicle
Observation period	:	
Result	:	negative

Method	:	other: OPPTS, OECD
Year	:	2001
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Attached document	:	This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The test article, a composite of three lots of commercial hexabromocyclododecane (HBCD), was administered by oral gavage in corn oil once daily to four groups of Crl:CD(SD)IGS BR rats (n=15/sex/group) at dose levels of 0 (control), 100 (low), 300 (mid) and 1000 (high) mg/kg/day seven days per week for 90 days. The dosage volume was 5 ml/kg. The control animals received the vehicle, corn oil, only. At the end of the 90-day treatment period, 10 animals/sex/group were euthanized and necropsied. The remaining rats continued on test untreated for a 28-day recovery period prior to necropsy. Functional Observational Battery and Locomotor Activity evaluations were performed on 5 animals/sex/group prior to study initiation, during the last week of test article administration (study week 13), and during the recovery period. No test article-related changes occurred in the Functional Observational Battery or Locomotor Activity evaluations at any time point. The NOELs for the Functional Observation Battery and the Motor Activity tests was 1000 mg/kg bw, the highest dose tested.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

15.12.2004 (42)

Endpoint	:	Neurotoxicity
Study descr. in chapter	:	5.4 Repeated Dose Toxicity
Reference	:	Chengelis C. 1996 A 28-day repeated dose oral toxicity study of HBCD in rats. Study No. WIL-186004. WIL Research Laboratories, Inc. Ashland, OH).
Type	:	other: Functional Observational Battery, Motor Activity
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral, gavage
No. of animals	:	24
Vehicle	:	other: corn oil
Exposure period	:	28 day(s)
Frequency of treatm.	:	once daily
Doses	:	0, 125, 350, 1000 mg/kg bw
Control group	:	yes, concurrent vehicle
Observation period	:	
Result	:	negative
Method	:	other: OECD
Year	:	1996
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Attached document	:	This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. HBCD, in the vehicle corn oil, was administered orally by gavage to three groups of Sprague-Dawley Crl: CD BR rats for a period of 28 consecutive

days. Dose levels were 125 (low), 350 (mid), or 1,000 (high) mg/kg/day, administered at dosage volume of 5 ml/kg. The test groups consisted of 6 males and 6 females in the 125 and 350 mg/kg/day groups and 12 males and 12 females in the 1000 mg/kg/day group. A concurrent control group comprised of 12 males and 12 females received the vehicle, corn oil, for 28 consecutive days at a dosage volume of 5 ml/kg. At the end of the dosing period, 6 animals/sex/group were sacrificed and necropsied. The remaining 6 animals/sex in the control and 1000 mg/kg/day groups remained on-test untreated for a 14-day recovery period. At the end of the recovery period, all animals were sacrificed and necropsied. Functional observational battery and motor activity evaluations were performed during weeks -1 (pretest), 3, and 5 (recovery). Functional observation battery and motor activity results from treated animals were compared statistically by sex and treatment day to their respective control groups ($p < 0.05$). These parameters were not affected by treatment with the test article. No statistically significant differences were observed between treated and control animals at any time point.

The NOELs in the Functional Observation Battery and the Motor Activity tests were 1000 mg/kg bw, the highest dose tested.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
15.12.2004 (43)

15.12.2004

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

Test substance : HBCD
Method : LCMS

Attached document : HBCD is susceptable to decomposition at temperatures > approximately 269 degrees Centigrade. TGA analysis of the commercial HBCD product indicates 50% weight loss at 269 degrees Centigrade and 90% weight loss at 274 degrees Centigrade. Thus, it is not ideally suited to gas chromatography because of the temperatures commonly employed in that methodology. Also, a gas chromatography method that is able to separate the three stereoisomers has not been identified. As a consequence, the preferred analytical method is liquid chromatography coupled with mass spectrometry. An LCMS method has been developed by Wildlife International LTD under the sponsorship of the ACC Brominated Flame Retardant Industry Panel that can separate and quantitate the three HBCD stereoisomers in various matrixes. (See the studies sponsored by BFRIP in 2003 and 2004.)

Reliability : (1) valid without restriction
29.12.2004

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

14.12.2004

10.3 RISK ASSESSMENT

November 18, 2005

Mr. Michael Gallagher
PBT Coordinator
State of Washington Department of Ecology
Box 47600
Olympia, WA 98504
MGAL461@ecy.wa.gov
FAX: 360-407-6884

RE: Inclusion of Hexabromocyclododecane (HBCD) in the Department of Ecology's Proposed Rule regarding Persistent Bioaccumulative Toxins (PBTs) (Chapter 173-333 WAC).

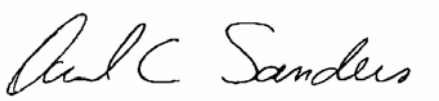
Dear Mr. Gallagher:

The Bromine Science and Environmental Forum (BSEF) is a global industry association composed of the major manufacturers of brominated flame retardants and our mission is to further the scientific understanding of our products. As such, BSEF and its member companies have sponsored numerous studies on the potential health and environmental effects of our products and have engaged the services of individuals with in-depth knowledge of the toxicology of our products. Additional studies of HBCD's potential for environmental persistence are on-going.

BSEF submits the attached comments on the inclusion of hexabromocyclododecane (HBCD) in the Department of Ecology's proposed rule regarding Persistent Bioaccumulative Toxins (PBTs) (Chapter 173-333 WAC).

HBCD does not meet the criteria set out for PBT substances in the Department's proposed rule, and should be deleted from the list of substances proposed for inclusion.

Sincerely,



Raymond B. Dawson, PhD.
Chairman
BSEF

David C. Sanders, PhD.
Director, North America
BSEF

Enclosure: Comments
HBCD IUCLID file

HEXABROMOCYCLODODECANE (HBCD)

The Department of Ecology proposes that a substance would be considered persistent, bioaccumulative and toxic if:

- Its half-life in water, soil or sediment is > 60 days;
- Its bioconcentration/bioaccumulation factor is > 1000; and
- It is a known carcinogen, reproductive or neurologic toxicant, has a reference dose (RfD) of < 0.003 mg/kg/d, or is toxic to fish on chronic exposure.

Our comments on the above proposed criteria are in a separate submittal.

HBCD does not meet the Department's proposed criteria for a persistent, bioaccumulative and toxic substance, and should be removed from the proposed rule. The data supporting its removal is presented below and in the attached IUCLID file.

Persistence
HBCD was determined not to be 'readily biodegradable' in the MITI test; e.g. it was not degradable by sewage microbes within a 28 day period when tested under stringent conditions. However, HBCD was degradable under more environmentally realistic conditions, and therefore should not be considered persistent.

Transformation in Aerobic and Anaerobic Water/Sediment Microcosms (Sponsor: ACC Brominated Flame Retardant Industry Panel and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. Its composition was 8.68% alpha, 6.12% beta, and 85.19% gamma. This study was performed according to Good Laboratory Practices and OECD Guideline 308.

The transformation of hexabromocyclododecane (HBCD) was determined in aerobic and anaerobic water/sediment microcosms based on the Organization for Economic Co-Operation and Development (OECD) Test Guideline 308 "Aerobic and Anaerobic Transformation in Aquatic Sediment Systems." Laboratory batch microcosms were prepared with authentic water and sediment collected from two rivers in the eastern United States. Aerobic microcosms were pre-incubated at 20 ± 1 °C for 49 days and maintained by periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen. Anaerobic microcosms were prepared in an anoxic atmosphere (70% N2, 28% CO2, and 2% H2). The microcosms were pre-incubated at 23 ± 1 °C for 43 to 44 days to allow the microcosms to stabilize. HBCD was then added to the microcosms at nominal concentrations ranging from 34 to 89 ng/g (sediment dry weight). Biologically inhibited (i.e., abiotic) controls were prepared by steam sterilization of the sediment/water mixture prior to the addition of HBCD. Microcosms were incubated in the dark at 20 ± 1 °C for 119 days. The concentration of HBCD in the microcosms was

determined at selected time intervals in the water and sediment phases utilizing high performance liquid chromatography-mass spectrometry (LC-MS). Aerobic microcosms were analyzed on days 0, 1, 7, 21, 64, 91, and 119, while anaerobic microcosms were analyzed on days 0, 1, 7, 14, 61(or 62), 91, and 119.

HBCD concentrations decreased over time in both the aerobic and anaerobic microcosms.

HBCD concentrations in the viable aerobic microcosms from both river systems decreased at least 90% within 21 days, while the corresponding decreases in the abiotic controls ranged from 7 to 62%. Disappearance of HBCD was observed in both the viable and abiotic anaerobic microcosms with the rate of loss more rapid in the viable microcosms, with HBCD reaching non-detected levels within 7 days. In contrast, HBCD concentrations in the abiotic controls decreased from 48 to 62% after 14 days. Pseudo-first order kinetics rate constants for the biotransformation of HBCD were determined by subtracting the abiotic rate constant from the viable rate constant. Biotransformation half-lives for HBCD in the two river systems were determined to be 11 and 32 days in the aerobic microcosms and 1.1 and 1.5 days in the anaerobic microcosms. Brominated degradation products were not detected in the sediment and water layers or in the headspace of the microcosms.

The purpose of this study was to determine the environmental lifetime of HBCD under realistic environmental conditions. Laboratory microcosms were used to evaluate the transformation of HBCD in aerobic and anaerobic water/sediment microcosms. Sediment degradation processes are expected to play a major role in determining the environmental lifetime of HBCD since, upon release into the environment, the majority of HBCD is likely to partition into the soil or sediment compartments. In this study, HBCD loss was observed in both viable and abiotic sediments although the rates were appreciable faster in the viable sediments. Biologically mediated transformation processes (i.e., biotransformation) accelerated the rate of loss of HBCD when compared to the biologically inhibited (i.e., heat-treated) microcosms. Brominated degradation products were not detected in any of the sediment microcosms.

Over the last several years a number of international protocols (EC 1996, UNECE 1998, UNEP 2000) have been put forth for the classification of chemicals as persistent (P), bioaccumulative (B), and toxic (T). The criteria for persistence in these initiatives includes half-lives in soil and sediments ranging from 120 to 180 days. In this investigation the resulting biotransformation half-lives for HBCD in the two river systems were determined to be 11 and 32 days in the aerobic sediments and 1.1 and 1.5 days in the anaerobic sediments, respectively.

Limited information is available for the reactions of HBCD in the environment. However, the aerobic degradation and mineralization of a similar type of cyclic aliphatic halogenated fire retardant, FR-651A (mixture of pentabromo-chlorocyclohexane, tetrabromo-dichlorocyclohexane, and tribromotrichlorocyclohexane) has been observed. A soil half-life of ~11 days, based upon disappearance of 14C-FR-651A from soil, was

reported. Complete degradation of 14C-FR-651A was also observed with a mineralization half-life on the order of 93 days.

An examination of the reactions of brominated aliphatic compounds that contain structural features similar to HBCD can also provide insight into the possible reaction pathways available for HBCD. Brominated aliphatic compounds are known to be susceptible to both hydrolytic and nuclelophilic attack. The reactivity of halogenated aliphatic compounds depends of the strength of the carbon-halogen bond, and increases in the order of F < Cl < Br. For example, the hydrolysis half-lives at pH 7 and 25 °C for methyl fluoride, methyl chloride, and methyl bromide are 1.1 x 104, 340, and 20 days, respectively. At environmental pH's neutral and base catalyzed hydrolysis reactions are most important, and depend on the degree and patterns of halogen substitution. Sulfur based nucleophiles can react rapidly with halogenated aliphatic compounds, thereby reducing their lifetimes in the environment. The half-life of 1-bromohexane in water at 25 °C was reduced from 20 days to approximately one day in 5 mM HS- or 0.07 mM polysulfide (Sx2-). Similarly, the half-life of 1,2-dibromoethane in water at 25°C was reduced from 1,000 days to 4 days in the presence of 5 mM HS-. Sulfide and polysulfides would be expected to be present in sediments at low redox potentials. Thus, reaction of HBCD with these sulfur-based nucleophiles may partly explain the loss of HBCD in the microcosm studies.

Halogenated aliphatic compounds are susceptible to reductive dehalogenation reactions in natural reducing environments. The rates of reactions vary depending on the strength and electron affinity of the carbon-halogen bond, and the stability of the carbon-radical species resulting from the initial electron transfer that occurs. Vicinal dehalogenation reactions are particularly important, and are dependent on the halogen atom. Half-lives for vicinal dehalogenation of 1,2-diiodoethane, 1,2-dibromoethane, and 1,2-dichloroethane are 0.6, 55, and 1.8 x 104 hours, respectively. The rapid disappearance of HBCD in the anaerobic sediment microcosms may be partly explained by reductive dehalogenation reactions. In addition, the disappearance of HBCD in the aerobic sediment microcosms may also be at least partly explained by reductive dehalogenation reactions. Anaerobic gradients often occur below the surface of sediments that are exposed to an aerobic water column. Such gradients would be expected to form in the static microcosms used in this study.

Based upon these results, HBCD is not persistent. Its half-lives in sediment are below the criteria specified by the various international protocols and specifically below the 120 days value specified in the European Commission's Technical Guidance Document on Risk Assessment (EC 1996).

Davis J, Gonsior S and Marty G. Evaluation Of Aerobic And Anaerobic Transformation Of Hexabromocyclododecane In Aquatic Sediment Systems. Study Number 021081. Environmental Chemistry Research Laboratory, Toxicology & Environmental Research and Consulting. The Dow Chemical Company Midland, Michigan. 2003.

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Davis J, Gonsior S and Marty G. Evaluation Of Aerobic And Anaerobic Transformation Of Hexabromocyclododecane In Aquatic Sediment Systems. Study Number 021081. Environmental Chemistry Research Laboratory, Toxicology & Environmental Research and Consulting. The Dow Chemical Company Midland, Michigan. 2003.

Transformation in Aerobic and Anaerobic Soil Microcosms (BFRIP and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to Good Laboratory Practices.

The transformation of HBCD was determined in aerobic and anaerobic soils based on the Organization for Economic Co-Operation and Development (OECD) Test Guideline 307 "Aerobic and Anaerobic Transformation in Soil." Soil microcosms were prepared by adding a sandy loam surface soil to serum bottles sealed with Teflon Ò coated septa. Aerobic microcosms were prepared by adjusting the soil moisture to 20% (by weight) and periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen. The microcosms were pre-incubated at 20 ± 1 °C for 35 days. Anaerobic microcosms were prepared in an anaerobic atmosphere (70% N2, 28% CO2, and 2% H2) by flooding the soil with water and pre-incubating the microcosms at 23 ± 1 °C for 43 days to allow low redox (e.g., methanogenic) conditions to develop. HBCD was then added to microcosms at a nominal concentration of 25 ng/g (soil dry weight), together with activated sludge (5 mg/g, dry weight basis) from a municipal wastewater treatment plant to simulate sludge land treatment applications. Biologically inhibited (abiotic) controls were prepared by steam sterilization prior to the addition of HBCD. Microcosms were incubated in the dark at 20 ± 1 °C for 119 days. The concentration of HBCD in the microcosms was determined at selected time intervals utilizing high

performance liquid chromatography-mass spectrometry (LC-MS). Aerobic microcosms were analyzed on days 0, 1, 7, 21, 48, 65, and 119, while anaerobic microcosms were analyzed on days 0, 1, 7, 21, 56, 91, and 119.

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Limited information is available for the reactions of HBCD in the environment. However, the aerobic degradation and mineralization of a similar type of cyclic, aliphatic halogenated fire retardant, FR-651A (mixture of pentabromochlorocyclohexane, tetrabromo-dichlorocyclohexane, and tribromotrichlorocyclohexane) was observed. A soil half-life of ~11 days based upon disappearance of 14 C-FR-651A from soil was reported. Complete degradation of 14C-FR-651A was also observed with mineralization half-life on the order of 93 days.

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Transformation in Aerobic and Anaerobic Soil Microcosms (Sponsor: ACC BFRIP and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to Good Laboratory Practices.

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<u>Investigation of the biodegradation of [14C]Hexabromocyclododecane in sludge, sediment, and soil (European Brominated Flame Retardant Industry Panel)</u>

This study investigated the biodegradation of the three HBCD stereoisomers, alpha, beta and gamma, and the identity of major degradation products. The formation and identification of degradation products of HBCD was assessed in activated and digester sludge, river sediment, and surface soil. Both aerobic and anaerobic biodegradation were evaluated in laboratory reaction mixtures and batch microcosms. This study was performed according to the relevant OECD Guidelines (302B; 307, 308), ISO 11734, and Good Laboratory Practices.

To generate sufficient levels of [^{14}C]degradation products for their identification, [14C]HBCD was added to reaction mixtures and microcosms at nominal concentrations ranging from 3 to 5 mg/kg (or mg/L), exceeding the water solubility of the test material by greater than an order or magnitude. Duration of the studies ranged from approximately 60 to 112 days. Reaction mixtures and batch microcosms were extracted and analyzed by high performance liquid chromatography (HPLC) with radiochemical detection to follow the degradation of the 3 stereoisomers and formation of [14C]products. Product identification was facilitated by analyses of extracts from the soil, sediment and sludge mixtures by HPLC-atmospheric pressure photo ionization-mass spectrometry (APPI-MS) or gas chromatography-electron impact ionization-mass spectrometry (GC-EI-MS).

Substantial biological transformation of [^{14}C]HBCD was observed in the anaerobic digester sludge and in freshwater aerobic and anaerobic sediment microcosms. Conversely, no degradation of HBCD was noted in the soil microcosms incubated under aerobic conditions. In the digester sludge and sediment, degradation of each of the three stereoisomers occurred over the course of the study. Little difference was noted in the disappearance of the three stereoisomers, indicating similarity in the extent of degradation of each isomer.

Concomitant with the loss of [^{14}C]HBCD in the sludge and sediment test mixtures was formation of three [^{14}C]products. Using a combination of HPLC-APPI-MS and GC-EI-MS these metabolites were identified as tetrabromocyclododecene, dibromocyclododecadiene, and cyclododecatriene. These products suggest HBCD is sequentially debrominated via dihaloelimination by naturally occurring microorganisms in wastewater sludge and aquatic sediment. During each sequential debromination, two bromines are lost from vicinal carbons with the subsequent formation of a double bond between the adjacent carbon atoms. These results demonstrate microorganisms naturally occurring in aquatic sediment and wastewater sludges can completely debrominate HBCD.

Davis JW, Gonsior SJ, Markham DA, and Marty GT. 2004. Investigation of the biodegradation of [14C]hexabromocyclododecane in sludge, sediment, and soil. Laboratory Project Study ID 031178. Toxicology & Environmental Research and Consulting. The Dow Chemical Company, Midland, MI.

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The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. Its composition was 8.68% alpha isomer, 6.12% beta isomer and 85.19% gamma isomer. This study was performed according to Good Laboratory Practices and OECD 307.

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Toxicity, Mammalian

HBCD is not acutely toxic, irritating or sensitizing. It is not mutagenic. Its no-adverse-effect-level in repeated dose studies is 1000 mg/kg/d. It is not a developmental or reproductive toxicant, with a no affect level of 1000 mg/kg/d.

The most relevant studies are summarized below. Additional studies can be found in the enclosed IUCLID file.

A 28-day repeated dose oral toxicity study of HBCD in rats. (Sponsor: ACC Brominated Flame Retardant Industry Panel)

This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc.

HBCD, in the vehicle corn oil, was administered orally by gavage to three groups of Sprague-Dawley Crl: CD BR rats for a period of 28 consecutive days. Dose levels were 125 (low), 350 (mid), or 1,000 (high) mg/kg/day, administered at dosage volume of 5 ml/kg. The test groups consisted of 6 males and 6 females in the 125 and 350 mg/kg/day groups and 12 males and 12 females in the 1000 mg/kg/day group. A concurrent control group comprised of 12 males and 12 females received the vehicle, corn oil, for 28 consecutive days at a dosage volume of 5 ml/kg. At the end of the dosing period, 6 animals/sex/group were sacrificed and necropsied. The remaining 6 animals/sex in the control and 1000 mg/kg/day groups remained on-test untreated for a 14-day recovery period. At the end of the recovery period, all animals were sacrificed and necropsied.

Animals were observed twice daily for mortality and morbidity. Clinical signs were recorded daily. Body weight and food consumption were measured weekly. Functional observational battery and motor activity evaluations were performed during weeks -1 (pretest), 3, and 5 (recovery). Samples for hematology and serum chemistry evaluations were collected at the primary (28 day) and recovery (42 day) sacrifices. Complete necropsies were performed on all rats. The brain, liver, kidney, heart, spleen, testes and epididymus or ovaries, adrenal glands, and thymus from all animals were weighed at each sacrifice. Approximately 40 tissues were collected and preserved at each necropsy from all animals. The following tissues were examined microscopically from the control and high dose animals: liver, kidney, heart, spleen, testes (males), prostate (males), seminal vesicles (males), epididymus (males), ovaries (females), adrenal glands, thymus, bone with marrow (sternebra), brain, stomach, cecum, duodenum, ileum, jejunum, lymph node, peripheral nerve (sciatic), spinal cord, lung, trachea, uterus (females), urinary bladder, and all gross lesions. The lungs, liver, kidneys, stomach, thyroid, gross lesions and target organs were examined in all dose levels.

Survival was not affected by administration of the test article. All animals survived to the scheduled sacrifice. Clinical signs observed during the study were nonspecific, low in incidence, non-dose-related and not considered related to test article.

Body weights, weight gain and food consumption of treated animals were compared statistically by sex and treatment day to their respective control groups ($p < 0.05$ or 0.01) and were not affected by treatment. No statistically significant differences in body weight between control and treated animals were detected with the exception of an increase in mean female body weight in the 350 mg/kg/day group during week 2 of treatment. Mean female body weight at that time point was 196 g versus 179 in the control group. No statistically significant differences in body weight gain between control and treated animals were detected with the exception of a decrease in mean male body weight gain in the 1,000 mg/kg/day recovery group during week 1 of recovery. Mean male body weight gain at that time point was 21 g versus 31 in the control group; mean male body weight was not statistically different from the control mean. No statistically significant differences in food consumption between control and treated animals were detected with the exception of an increase in mean female food consumption in the 350 mg/kg/day group during weeks -1, 1, and 2 of treatment. Mean female food consumption at that those time points were 18, 17 and 17 g versus16, 15 and 15 g in the control group, respectively.

Functional observation battery and motor activity results from treated animals were compared statistically by sex and treatment day to their respective control groups ($p < 0.05$). These parameters were not affected by treatment with the test article. No statistically significant differences were observed between treated and control animals at any time point.

No statistically significant differences between treated and control animals were found for hematology parameters with the exception of an increase in the mean activated partial thromboplastin time in the 1000 mg/kg/day males on week 4 and a decrease in the mean prothrombin time in the 1000 mg/kg/day females on week 4. These statistical differences were not of toxicological significance.

No toxicologically significant effects on serum chemistry values related to test article administration were observed at the 28-day primary and 42-day recovery sacrifice. Scattered instances of statistically significant differences between treated and control animals were detected for some serum chemistry parameters at the 28-day primary sacrifice. These scattered statistical differences were not considered toxicologically significant because the statistical differences occurred: in the absence of a dose response, in the absence of the accompanying clinical chemistry changes expected, in the opposite direction from what occurs in a toxic state, in a direction which is without physiologic significance, or due to potential interference with the laboratory method. No statistically significant differences in serum chemistry parameters were detected between groups at the 42-day recovery sacrifice.

No gross lesions that could be attributed to the test article were detected at either necropsy. Gross lesions were nonspecific, low in incidence, non-dose-related and considered incidental.

No microscopic lesions that could be attributed to the test article were detected on histopathologic exam. Microscopic changes were nonspecific, low in incidence, non-dose-related and considered incidental.

No statistical significant differences in organ weight or organ to body weight ratios were detected between control and treated animals with one exception. Absolute liver weights were statistically significantly increased with respect to control means at the 28-day sacrifice in males in the high dose and females in the mid and high dose. Liver to body weight ratios in mid and high dose males and low, mid and high dose females were statistically significantly increased at the 28-day sacrifice. At the recovery sacrifice, male absolute and liver to body weight ratio were statistically comparable to the control mean whereas female absolute liver weights and liver to body weight ratio were statistically significantly increased with respect to control mean. The difference in absolute liver weight between control and treated females was less pronounced at the end of the recovery period, indicating the increase in liver weight was reversible in females as well as males. In the absence of test article related histopathologic and serum chemistry changes, increases in liver weight are considered an adaptive, rather than a toxic response, are not uncommon in the rat, and are most likely the result of microsomal induction.

In conclusion, no systemic toxicity was observed at any dose level. Based on the results of this study, the NOAEL (No Observed Adverse Effect Level) of HBCD administered orally to male and female rats for 28 consecutive days was 1,000 mg/kg/day.

Chengelis C. 1996. A 28-day repeated dose oral toxicity study of HBCD in rats. Study No. WIL-186004. WIL Research Laboratories, Inc. Ashland, OH.

<u>An Oral (Gavage) 90 Day Toxicity Study of HBCD in Rats. (Sponsor: ACC Brominated Flame Retardant Industry Panel)</u>

This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc.

The test article, a composite of three lots of commercial hexabromocyclododecane (HBCD), was administered by oral gavage in corn oil once daily to four groups of Crl:CD(SD)IGS BR rats (n=15/sex/group) at dose levels of 0 (control), 100 (low), 300 (mid) and 1000 (high) mg/kg/day seven days per week for 90 days. The dosage volume was 5 ml/kg. The control animals received the vehicle, corn oil, only. At the end of the 90-day treatment period, 10 animals/sex/group were euthanized and necropsied. The remaining rats continued on test untreated for a 28-day recovery period prior to necropsy.

In addition to the main toxicology groups, two satellite groups of 20 animals/sex/group were treated concurrently in an identical manner at dose levels of 0 or 1000 mg HBCD/kg/day for up to 90 days. Body weights were recorded weekly. Two animals/sex/group were euthanized on study days 2, 6, 9, 13, 20, 27, 55, 89, 104 and 118, and blood and body fat (mesenteric and/or omental) were collected. The body fat was analyzed for HBCD content.

Animals in the main toxicology groups were observed twice daily throughout the study for mortality and morbidity. Body weights and food consumption were measured weekly. Blood was collected at study weeks 3 (n=5/sex/group), 13 (n=10/sex/group) and 17 (n=5/sex/group) for hematology, serum chemistry and hormone (T3, T4 and TSH) measurements. Urine was collected prior to each necropsy, at study weeks 13 and 17, for urinalysis. Ocular examinations were performed prior to study initiation and during study weeks 12 and 15. Functional Observational Battery and Locomotor Activity evaluations were performed on 5 animals/sex/group prior to study initiation, during the last week of test article administration (study week 13), and during the recovery period. An examination of vaginal cytology (for estrus cycle determinations) was performed on study days 69-90. At each necropsy, sperm motility/viability, morphology, and number were assessed. Complete necropsies were performed on all animals. Approximately 40 organs or tissues/animal were collected and preserved. The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, thyroids with parathyroids, and uterus with cervix were weighed. Paraffin sections of tissues stained with hematoxylin and eosin from the control and 1000 mg/kg/day dose groups and the liver, lungs and thyroid glands in the 100 and 300 mg/kg/day doses, and gross lesions from all animals were examined under the light microscope. Livers from five randomly chosen animals/sex from the control and 1000 mg/kg/day dose groups were examined microscopically using Oil Red O or periodic acid Schiff's (PAS) reagent for evidence of lipid accumulation or glycogen accumulation/depletion, respectively. Statistical comparisons by sex and treatment day were made between the control and treated animals where indicated ($p<0.05$).

No test article-related effect on mortality occurred. Clinical signs were non-specific, low in incidence, non-dose-related and not related to test article administration. No test article-related changes occurred in body weight, food consumption, Functional Observational Battery or Locomotor Activity. No test article-related effects on hematologic parameters were noted. No test article-related ocular lesions were detected at the ophthalmic exams. No test article-related changes were noted on the estrus cycle as determined by vaginal cytology, or on sperm motility/viability, morphology, and number. Instances of statistically significant differences between control and some treatment groups were detected at study week 13 in the clinical chemistry data, hormone data, organ weight data and histology findings. They were considered secondary to hepatic enzyme induction or were otherwise not considered adverse effects of treatment as discussed further below.

Statistically significant ($p<0.05$) test article-related serum clinical chemistry changes at week 13 include an increase in albumin (all dose levels for males), total protein (all dose levels for females and 1000 mg/kg/day for males), globulin (300 and 1000 mg/kg/day for females), and chloride (all doses for both sexes). In addition, increased gamma glutamyltransferase levels were noted in the 1000 mg/kg/day group ($p<0.05$). Thyroxine (T4) levels were decreased at study week 13 compared to the control mean in all male dose groups and the 300 and 1000 mg/kg/day dose females ($p<0.05$). There were no corresponding statistical effects on T3 and TSH.

While potentially test article-related, the changes in serum chemistry parameters were not of sufficient magnitude to be adverse, occurred in otherwise clinically normal animals, tended to be within or close to historical control values, and were not present at the end of the recovery period. The increased serum albumin and gamma glutamyltransferase levels were considered related to the increased in liver weight in treated animals that was believed due to enzyme induction. Hepatic GGT is inducible in the rat (Teschke et al 1983. Gut. 24(7):625-30; Chandar and Nagarajan 1984. Biochem Int. Jan;8(1):41-8), and this induction has been shown to increase serum GGT levels in the rat (Goldberg 1980. Crit Rev Clin Lab Sci. 12(1):1-58; Teschke et al. 1983. Gut 24(7):625-30; Nishmura and Teschke 1982. Biochem Pharmacol. Feb 1;31(3):377-81; Satoh et al. 1982. J Pharmacol Exp Ther. Jun;221(3):795-800). The increase in serum chloride was considered secondary to be presence of free bromide in the test article preparation that interfered with the chloride determination methodology. The decrease in T4, which was also reversible, was considered related to the increased liver weights in that induction of hepatic UDPG is known to enhance the elimination of T4. The rat is particularly sensitive to hepatic enzyme induction.

The incidence of observations noted at gross necropsy was low and was not accompanied by evidence of frank organ damage. On histopathologic examination, mild findings were detected in both the control and treated groups. Potential test article-related histologic changes were identified in the liver and thyroid glands, but these were not considered to be indicative of frank toxicity. The liver changes in male rats at the 90-day necropsy (Study Week 13) were characterized as minimal hepatocellular vacuolation and occurred in 10% of control males and ~50% of the males at 100, 300 and 1000 mg/kg/day. Thus, there was no increase in incidence with dose. Minimal hepatocellular vacuolation was also detected in females in the control and treated groups without a clear dose response (3 to 4/10 animals per group). Mild and moderate vacuolation was detected in females only in the 300 (1/10) and 1000 mg/kg/day (2/10) dose groups. Minimal to mild hepatocellular hypertrophy was also detected only in the 1000 mg/kg/day group (5/10) females. Minimal thyroid follicular cell hypertrophy was detected 1/10, 1/10, 5/10 and 7/10 males in the control, 100, 300 and 1000 mg/kg/day groups, respectively and in 4/10 and 3/10 females in the 300 and 1000 mg/kg/day groups respectively. In addition, mild thyroid follicular hypertrophy was detected in 4/10 females in the 1000 mg/kg/day group.

The histologic changes in the liver were accompanied by an increase in liver weight. In contrast there were no statistically significant changes in thyroid weight (absolute, relative to body weight and relative to brain weight). At study week 13, mean liver

weights in all dose levels of both sexes (absolute, relative to body weight and relative to brain weight) were increased compared to the male and female control means ($p<0.05$).

The changes seen in this study, an increase in liver weight, the lack of adverse histologic effects in the liver and the apparent normal functioning of the liver, are consistent with enzyme induction (Amacher et al. 1998. Food Chem. Toxicol. 36:831-830), and hepatic enzyme induction is an adaptive, and not an adverse, effect (Williams and Iatropoulos 2002. Toxicol Pathol. Jan-Feb;30(1):41-53). Hepatocellular vacuolation and hypertrophy, seen in this study, often accompany the increased liver weight caused by liver enzyme induction, and were reversible. At week 17, the liver changes (weight and histology) had at least partially, if not fully, resolved in all treated groups without delayed or long-term toxic effects.

Limited pharmacokinetic studies indicate HBCD is extensively metabolized prior to excretion in the feces and urine. The results of the fat analysis in this study indicate that mammalian system handles the 3 HBCD stereoisomers differently, and may be less efficient at eliminating one stereoisomer over another. (The relative isomer concentrations in adipose tissue at all time points were alpha>>gamma>beta in contrast to the test article composition of gamma>>alpha>beta.) Thus, it should not be unexpected that hepatic enzyme induction occurs with exposure to substantial doses over a significant period of time, as was the case in the 90-day study.

The histologic changes in the thyroid consisted of a slight increase in the incidence of minimal follicular cell hypertrophy in the high dose males and minimal or mild hypertrophy in the high dose females. It was not readily apparent that these minimal changes were an effect of treatment, and in any event appeared reversible. The follicular cell hypertrophy may have been related to serum T4 levels. Follicular cell hypertrophy is the normal physiological response to reduced to serum T4 levels and is the typical adaptive response of a healthy normally functioning organism acting to maintain serum T4 levels in the normal range.

The mean prostate weight was increased in the 1000 mg/kg/day group males at the primary necropsy. This was not considered to be of toxicological significance since the increase did not persist to the recovery period, there were no correlating histologic findings and no change in seminal quality.

HBCD was detected in the adipose tissue of male and female rats treated with 1000 mg/kg/day for up to 90 days. Isomer-specific analysis showed that the relative isomer concentrations in adipose tissue at all time points were alpha>>gamma>beta, which is in contrast to the test article composition (gamma>>alpha>beta). Steady state levels were achieved by study day 27. Levels in male and female rats were similar at all time points and declined during the recovery period.

All the test article-related changes at 100 and 300 mg/kg/day were mild, reversible, generally secondary to hepatic enzyme induction (which is an adaptive not a toxic change) and without effect on the clinical condition of the animals. The findings

observed at 1000 mg/kg/day (increased serum gamma glutamyltransferase, increased liver and prostate weights), were also reversible, not associated with specific target organ damage or diminished function and were, therefore, of limited, if any, toxicologic significance. On this basis, the no-observed-adverse-effect level (NOAEL) of HBCD administered to Crl:CD®(SD)IGS BR rats by gavage in corn oil for 90 days is 1000 mg/kg/day.

Chengelis C. An Oral (Gavage) 90 Day Toxicity Study of HBCD in Rats. Study No. WIL-186012. WIL Research Laboratories, Inc., Ashland, Ohio. 2001.

A Prenatal Developmental Toxicity Study of Hexabromocyclododecane (HBCD) in Rats. (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The study was performed according to EPA, OECD and GLP guidelines. This study was required by KEMI (without consultation of the EU Technical Meeting) because KEMI decided the existing study in the literature (Murai et al.) was insufficient.

HBCD was administered in corn oil by gavage to 25 presumed pregnant Crl:CD(SD)IGS Br rats/group once daily from gestation days 6-19 at doses of 0, 250, 500 or 1,000 mg/kg/day. Control animals received corn oil only. Female rats were mated in-house and were treated daily on gestation days 6-19 with HBCD via gavage at dose levels of 0 (vehicle control), 250, 500 or 1000 mg/kg/day at a constant volume of 5 ml/kg. Individual doses were based on the most recent body weight. The day on which evidence of mating was observed was considered day 0 of gestation. Dams were observed daily and maternal body weight and food consumption measured at appropriate intervals. Females were euthanized on day 20 of gestation and necropsied. Gravid uterine and liver weights were recorded. Litters were delivered by cesarean section. The total number of corpora lutea, total number of implantations, early and late resorptions, number and location of all fetuses, and the sex and individual weights of fetuses were recorded. All fetuses were examined grossly. Approximately one-half of the fetuses in each litter were stained with Alizarin Red S and Alcian Blue and evaluated for skeletal/cartilaginous malformations and ossification variations. The maternal day 20 gestation examinations and cesarean sections, and subsequent fetal evaluations were performed blind to treatment.

No mortality occurred during the course of the study. No treatment-related clinical signs were observed. Body weight gain and food consumption were not adversely affected. No treatment-related findings were detected at necropsy. Intrauterine growth and survival were unaffected by treatment. No treatment-related fetal malformations or developmental variations were observed. The no-effect level (NOEL) for maternal toxicity and developmental toxicity was 1,000 mg/kg/day, the highest dose tested

Stump, D. 1999. A Prenatal Developmental Toxicity Study of Hexabromocyclododecane (HBCD) in Rats. Laboratory Study No.: WIL-186009. WIL Research Laboratories, Inc., Ashland, OH.

Murai et al. 1985 (Pharmacometrics (Japan) 29(6):981-986)

Murai et al. 1985 (Pharmacometrics (Japan) 29(6):981-986) identified no reproductive or developmental effects in the rat at doses up to 1% in the diet administered from days 0-20 of gestation. This dose is approximately equivalent to 500 mg/kg/d.

The Murai et al study consisted of a 7 day dose range finding study (n=5 rats/dose group) and a combined teratogenicity-developmental study (n=20/dose group). Doses in the 7 day range finding study were 0, 0.3, 1, 3 or 10 g/kg/day. Doses as high as 10 g/kg/day produced no evidence of toxicity. A statistically significant ($P<0.01$) increase in liver weight was noted in groups receiving > 1 g/kg/day. Doses for the combined teratogenicity-developmental study were based on this increase in liver weight. In the combined teratogenicity-developmental study, pregnant female rats were fed diets containing 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation. Daily doses were estimated by the authors to be 0, 5, 50 or 500 mg/kg/day and the average total dose/rat/group was estimated to be 0, 0.13, 1.28 or 12.0 g/kg. Rats were observed daily and body weight and food consumption measured. Fourteen rats from each group were sacrificed on day 20 of gestation and their fetuses were examined for toxicity or teratogenicity. Approximately 150 fetuses/dose level were examined for evidence of teratogenicity. All fetuses from all litters were examined for signs of external anomalies. Approximately 2/3 of the fetuses/dam were examined for skeletal abnormalities; the remaining fetuses from each dam were examined for any abnormalities of the internal organs. In addition, six rats from each group were allowed to deliver their litters and growth of the litters was observed until the 7th week post-parturition.

The authors' estimated the doses in the feed were equivalent to 0, 5, 50 or 500 mg HBCD /kg body weight /day. No adverse effects were detected in any treatment group with respect to maternal weight gain, food consumption, or gross appearance of internal organs. The mean liver (absolute and relative to body weight) weight in the 1% group was statistically different (higher) from the control mean. Normal development was seen in neonates carried through to six weeks of age.

There was no adverse effect of treatment on the number of corpora lutea, implants, resporptions, live fetuses, sex ratio, or body or placental weight. No fetal deaths occurred in any group. No external, skeletal or visceral malformations were detected. A few skeletal variations were detected but where of similar types and numbers in the control and treated groups.

There were no significant differences between the control and treated groups in the number of implantation, live newborns, dead newborns, live newborn parturition index.

The weaning and survival index was comparable in the control and treated groups. Body weight changes in the newborns was comparable in all groups.

No reproductive or developmental effects where detected in rats at HBCD doses up to 1% in the diet (~500 mg/kg/d) administered from days 0-20 of gestation. Further, normal development was seen in neonates carried through to six weeks of age.

Dose levels: 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation [Murai estimate: 0, 5, 50 or 500 mg/kg/day]. No teratogenic effects. Normal development in neonates carried through age 6 wks. NOEL = 1% of diet.

Murai, T. Kawasaki, H., Kanoh, S. 1985. Studies on the toxicity of insecticides and food additives in pregnant rats - fetal toxicity of Hexabromocyclododecane. Pharmacometrics (Japan) 29(6):981-986).

Toxicity to Reproduction

Two teratology studies on HBCD are available; one published in the literature (Murai et al. 1988; high dose = 1% of the diet) and one recently completed by industry (1999) under current guidelines and Good Laboratory Practices using the HBCD in commercial production and use (high dose = 1,000 mg/kg/d). Both studies are negative for developmental toxicity. Repeated dose studies (two 28 day studies, one 90 day study, and one 18 month study in a second species) indicate HBCD does not affect the reproductive organs at doses up to 1,000 mg/kg/day. According to the OECD SIDS Manual, when teratology and 90 day studies show no effects on the reproductive system then the requirement for the reproductive endpoint are met. Teratology, 28 day, 90 day and 18 month studies all demonstrate HBCD has no effect on the reproductive system at the limit dose of 1,000 mg/kg/d.

In the 2001 90-Day study, considered the most informative of the subchronic studies mentioned in the preceding paragraph, the following organs of the reproductive tract were weighed at the 90-day and recovery sacrifices: epididymides (total and cauda), ovaries (with oviducts), prostate, testes, and uterus and cervix. These organs plus the semininal vesicles, vagina and vas deferens were examined histologically in animals in the control and high dose groups. Vaginal smears for determination of the stage of estrus were obtained form all females once daily beginning study day 69 through the 90-day necropsy. An assessment of spermatogenesis was performed at the 90-day sacrifice by evaluating sperm motility/viability, morphology and epidiymal and testicular sperm numbers and production rate.

No adverse effect on the estrous cycle was detected in females receiving doses as high as 1,000 mg/kg/d for 90 consecutive days. No adverse effect on spermeogenesis was detected in males receiving doses as high as 1,000 mg/kg for 90 days. No test-article related changes in weight or microscopic effects were noted in the organs of the reproductive tract with the sole exception of an increase in weight of the prostate at 1,000

mg/kg/d on day 90. Mean prostate weight was increased in the 1,000 mg/kg/d dose group compared to the control mean after 90 days of treatment (0.95, 0.99, 1.12, 1.25* g in the control, 100, 300, and 1,000 mg/kg/d, respectively). The mean prostate weight relative to mean body and brain weights was also statistically increased at the high dose on day 90. At the recovery sacrifice, the prostate weights in the control and high dose groups were not statistically different. No test article-related changes in the prostate were detected on microscopic exam at either sacrifice.

Toxicity, Fish Chronic

HBCD was not chronically toxic to fish or daphnia at the limits of its aqueous concentration, based on the gamma isomer.

Fish Early Life Stage In Rainbow Trout (Oncorhynchus mykiss) (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current OECD guidelines and Good Laboratory Practices.

Rainbow trout embryos were exposed to nominal HBCD water concentrations of 0.43, 0.85, 1.7, 3.4 and 6.8 ug/L. The top two doses represent gamma HBCD's water solubility (3.4 ug/L) and two times gamma HBCD's water solubility (6.8 ug/L). A negative control and solvent control group were also included. Mean measured concentrations (LC/MS with heated nebulizer operated in the selective ion monitoring mode) were 0.25, 0.47, 0.83, 1.8 and 3.7 ug/L. This method was designed to monitor for all 3 HBCD diastereomers; however, the trace residues of the alpha and beta diastereomers evident in the water samples were below the established limits of quantitation. Comparison of the chromatograms from study initiation through study termination showed that the relative distribution of the HBCD diastereomers remained constant during the definitive study, and the gamma diastereomer measured results were consistent throughout the study.

Hatching success, time to hatch, time for larvae to swim-up, and post-hatch growth and survival were evaluated during the 88-day test. Rainbow trout exposed to HBCD at mean measured concentrations up to 3.7 ug/L (nominal concentration = 6.8 ug/L or twice HBCD's water solubility) for a 27-day hatching period and 61 days post-hatch showed no effects on hatching success, time to swim-up, larval survival, fry survival or growth. Consequently, HBCD was not chronically toxic to rainbow trout at concentrations at or above its limit of solubility. The NOEC for this study was 3.7 ug/L or 6.8 ug/L nominal (twice gamma HBCD's water solubility). The low-effect-concentration (LOEC) and maximum acceptable toxicant concentration (MATC) could not be determined due to absence of toxicity, but were considered >3.7 ug/L or >6.8 ug/L nominal (> twice gamma HBCD's water solubility).

Drottar et al. 2001. Hexabromocyclododecane (HBCD): An early life-stage toxicity test with the rainbow trout (Onchorhynchus mykiss). Project No.: 439A-112. Wildlife International, Ltd. Easton, MD.

Daphnia magna Life Cycle (21 Day) (Sponsor: ACC Brominated Flame Retardant Industry Panel)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

Nominal test concentrations were 0.85, 1.7, 3.4, 6.8 and 13.6 ug HBCD/L water; dose levels were based on gamma HBCD's water solubility, 3.4 ug/L. Measured test concentrations (LC/MS with negative ion atmospheric pressure ionization) were 0.87, 1.6, 3.1, 5.6 and 11 ug HBCD/L water (based on the gamma stereoisomer).

No statistically significant effects on survival, reproduction or growth of Daphnia magna were seen at HBCD concentrations < 3.1 ug/L (measured). Thus, HBCD's no effect concentration (NOEC), based on survival, reproduction and growth, to daphnia magna for 21 days was equivalent to HBCD's water solubility. The measured NOEC in this study was 3.1 ug/L and corresponded to a nominal HBCD concentration of 3.4 ug/L, e.g. gamma HBCD's water solubility. The lowest observed effect concentration (LOEC) and the maximum acceptable toxicant concentration (MATC) based on survival, growth and reproduction were greater than HBCD's water solubility. The LOEC, 5.6 ug/L, corresponded to nominal concentrations twice gamma HBCD's water solubility. The effect seen at this dose level was a reduction in length. Survival and reproduction at the 5.6 ug/L dose level were not affected. The MATC, 4.2 ug/L, was calculated as the mean of the NOEC and the LOEC. The MATC was greater than gamma HBCD's water solubility.

Drottar K. and Krueger H. 1998. Hexabromocyclododecane (HBCD): Flow-through life-cycle toxicity test with the cladocerna (Daphnia magna). Project No.: 439A-108. Wildlife International, Ltd. Easton, MD.